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(71) Applicant: THE UNITED STAES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Technology Transfer, Office of Technology Transfer, Box OOT, Bethesda, MD 20892 (US).

(72) Inventors: STANLEY, John, R. ; 10814 Old Coach Road, Potomac, MD 20854 (US). AMAGAI, Masayuki ; Grosvenor Tower, Apt. No. 1803, Bethesda, MD 20852 (US). KLAUS-HOVTUN, Vera ; 9424 Old Georgetown Road, Bethesda, MD 20814 (US).

(74) Agents: SCOTT, Watson, T. et al.; Cushman, Darby & Cushman, 1100 New York Avenue, N.W., Washington, DC 20005 (US).

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(54) Title: NUCLEOTIDE AND AMINO ACID SEQUENCE OF PEMPHIGUS VULGARIS ANTIGEN AND METHODS OF USE

(57) Abstract

Pemphigus vulgaris (PV) is a life-threatening skin disease in which autoantibodies against a keratinocyte cell surface 130-kD glycoprotein, PV antigen (PVA), cause loss of cell-to-cell adhesion with resultant epidermal blisters. The present invention relates to DNA sequences encoding the entire amino acid sequence of PVA. The invention also relates to recombinant constructs containing the DNA sequence for PVA, and host cells transformed therewith. In addition, the invention relates to methods of diagnosing and treating persons afflicted with PV disease.

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NUCLEOTIDE AND AMINO ACID SEQUENCE OF PEMPHIGUS VULGARIS ANTIGEN AND METHODS OF USE

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates, in general, to the molecular cloning and expression of a glycoprotein, and, in particular, to pemphigus vulgaris antigen (PVA) which is involved in the autoimmune skin disease pemphigus vulgaris (PV). The invention further relates to a cDNA sequence encoding PVA, to a recombinant DNA molecule that includes such a sequence and to cells transformed therewith.

Background Information

Pemphigus vulgaris (PV) is an autoimmune disease of skin and mucous membranes in which autoantibodies against the keratinocyte cell surface cause loss of cell-to-cell adhesion and blister formation (Stanley, 1989). PV antigen (PVA), which is defined by autoantibodies from these patients, has been characterized by immunoprecipitation and immunoblotting as a 130-kD glycoprotein (Stanley et al., 1982, 1984; Eyre and Stanley, 1988; Jones et al., 1986; Hashimoto et al., 1990). All patients with PV, but not normals or other disease control patients, have antibodies that bind this glycoprotein. More recent studies (Korman et al., 1989) have shown that in extracts of normal human epidermis PVA is linked by disulfide bonds to plakoglobin, an 85-kD molecule found in the plaque of the desmosome and cell-to-cell adherens junction (Cowin et al., 1986). Immunoelectron microscopic studies have shown that, although PVA is present in

desmosomes, it is probably also present as well along the entire cell surface of keratinocytes (Wolff and Schreiner, 1971; Jones et al., 1986b).

Several observations and studies have demonstrated that the autoantibodies from PV patients are pathogenic: 1) Disease activity in PV patients often correlates with anti-cell surface antibody titer, as determined by indirect immunofluorescence (Sams and Jordon, 1971). 2) Neonates of mothers with PV may have transient disease due to maternal IgG which crosses the placenta (Merlob et al., 1986). As maternal antibody is catabolized, disease subsides. 3) PV IgG alone, without complement or inflammatory cells, can cause loss of cell-to-cell adhesion, with the same histology as seen in PV blisters, in skin organ culture (Schiltz and Michel, 1976; Hashimoto et al., 1983). 4) Passive transfer of PV IgG to neonatal mice results in loss of cell-to-cell adhesion and blisters with typical PV histology (Anhalt et al., 1982).

SUMMARY OF THE INVENTION

Because PV autoantibodies cause loss of cell adhesion, we speculated that PVA might be a cell adhesion molecule (Jones et al., 1986b). To address this question, we cloned the cDNA encoding PVA using patients' antibodies. We used affinity-purified PV IgG to isolate cDNA, containing the entire coding sequence for PVA, from human keratinocyte expression libraries. Northern analysis indicated PV mRNA expression only in stratified squamous epithelia. The deduced amino acid sequence of PVA was unique but showed significant homology to members of the cadherin

family of Ca^{2+}-dependent cell adhesion molecules, most markedly to desmoglein I. These findings demonstrate that a novel epithelial cadherin is the target of autoantibodies in PV, a disease of epidermal cell adhesion. The DNA sequence and clones can be used for diagnostic purposes. For example, pemphigus vulgaris antigen proteins have been made from the cDNA and these proteins have been used to raise antibodies. These proteins can also be used in ELISA assays for detection of autoantibodies to diagnose pemphigus vulgaris. These sequences could also be used for specific therapy by using proteins derived from them for specific plasmapheresis.

Accordingly, it is an object of the present invention to provide a DNA fragment that encodes pemphigus vulgaris antigen.

It is another object of the present invention to provide an amino acid sequence for the pemphigus vulgaris antigen.

It is a further object of the present invention to provide a recombinantly produced, biologically stable pemphigus vulgaris antigen glycoprotein.

It is yet another object of the present invention to provide a recombinant DNA construct comprising a vector, and the above-described DNA fragment.

It is a further object of the present invention to provide a host cell transformed with the above-described recombinant DNA construct.

It is another object of the present invention to provide a method of producing pemphigus vulgaris antigen which comprises culturing a host cell under conditions such that the above-

described DNA fragment is expressed and pemphigus vulgaris antigen is thereby produced, and isolating pemphigus vulgaris antigen.

It is a further object of the present invention to provide an antibody to the above-described recombinant pemphigus vulgaris antigen.

It is another object of the present invention to provide a method of detecting the presence of pemphigus vulgaris antigen in a sample comprising the steps of contacting the sample with the above-described antibody, and detecting the presence or absence of a complex formed between the pemphigus vulgaris antigen and the antibody.

It is yet another object of the present invention to provide a method for the diagnosis of pemphigus vulgaris disease comprising the steps of:

(i) coating a surface with all, or a unique portion, of the above-described recombinantly produced pemphigus vulgaris antigen,

(ii) contacting the coated surface with serum from an individual suspected of having the disease; and

(iii) detecting the presence or absence of a complex formed between the pemphigus vulgaris antigen and antibodies specific therefor present in the serum.

It is a further object of the present invention to provide a diagnostic kit comprising a recombinantly produced pemphigus vulgaris antigen and ancillary reagents suitable for use in detecting the presence of antibodies to pemphigus vulgaris antigen in mammalian serum or tissue samples.

It is an object of the present invention to provide a therapeutic method for the treatment of pemphigus vulgaris disease comprising performing

plasmapheresis on an individual having pemphigus vulgaris disease, wherein the above-described recombinantly produced pemphigus vulgaris antigen is contacted with the individual's blood prior to reinfusion of the blood into the individual.

Other objects of the present invention will be apparent by the description of the embodiments that follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. PVA immunoprecipitated from keratinocytes cultured with (+) and without (-) tunicamycin. Immunoprecipitations were performed with either PV or, as controls, normal (N) sera. Arrow shows 130-kD glycosylated PVA. Arrowhead shows that PVA precipitated from extracts of cells cultured with tunicamycin, which inhibits N-glycosylation, migrates faster, at about 115 kD. (The bars on the right indicate migration of molecular weight standards, 200, 116, 97, and 66 kD).

Figure 2. Immunofluorescence of PVA on monkey esophagus.
(A) PV IgG affinity-purified on immunoblots of the 130-kD PVA. (B) PV IgG affinity-purified by epitope selection on the fusion protein produced by clone MJ315. (C) Control for B, epitope selection of PV serum by irrelevant clones. (D) Rabbit antibodies raised against the MJ315 fusion protein.
(Magnification x 136).

Figure 3. Immunoblot of NHEK extracts with PV serum and PV IgG affinity-purified on the 130-kD PVA and on the MJ315 clone.
Lane 1: PV serum binds the 130-kD PVA (arrowhead) as

well as other bands. Lane 2: PV IgG affinity-purified on PVA binds only the 130-kD PVA. Lane 3: PV IgG affinity-purified by epitope selection on clone MJ315 binds PVA. Lane 4: control for lane 3, epitope selection of PV serum by irrelevant clones does not result in binding on the immunoblot. (Molecular weight standards, indicated by bars are 200, 116, and 97 kD).

Figure 4. Specificity of PV binding to immunoblot of the MJ315 fusion protein.
Immunoblot of the MJ315 fusion protein produced in pUEX 1 with PV, pemphigus foliaceous (PF), bullous pemphigoid (BP), and normal (N) sera. Only PV sera bind the fusion protein (arrow). (Molecular weight standards indicated by bars are 200, 116, and 97 kD).

Figure 5. Northern analysis of PVA.
Northern blots of poly(A)$^+$ RNA with MJ315 cDNA (lanes 1-17) and human β-actin cDNA (below lanes 1-10, 13-17, to show relative amounts of RNA on each lane). Lane 1-NHEK; lanes 2,3-cultured human fibroblasts. The major mRNA for PVA is approximately 6 kb (arrow), and minor bands at approximately 4 and 3.5 kb are also seen. Lane 4-NHEK, positive control for PVA mRNA; lane 5-human brain; lane 6-human heart; lane 7-human lung; lane 8-human liver; lane 9-human kidney; lane 10-human placenta. (Lanes 4-10 were exposed for 15 hr, and corresponding actin lanes were exposed for 2 hr. Even when exposed for 72 hr, lanes 5-10 did now show PVA mRNA). Lane 11-monkey esophagus and lane 12-monkey tongue show PVA mRNA (approximately 6 kb) in these stratified squamous epithelia. Lane 13-monkey tongue, positive

control for PVA mRNA; lane 14-monkey liver; lane 15-monkey lung; lane 16-monkey small intestine; lane 17-monkey kidney. (Lanes 13-17, and corresponding actin lanes, exposed for 8 hr. Although the actin mRNA loading is light for monkey liver and lung, even with exposures up to 72 hr, these tissues do not show PVA mRNA). Lines to right of lanes 3 and 12 indicate RNA standards of 9.5, 7.5, 4.4, 2.4, and 1.4 kb.

Figure 6. Southern analysis of PVA.
Southern blot of human placental DNA digested with indicated restriction enzymes and hybridized to MJ315. (DNA size markers, indicated by bars, are 9.4, 6.6, 4.4, 2.3, and 2.0 kb).

Figure 7. Nucleotide and predicted amino acid sequence of PVA.
The putative signal sequence and transmembrane domain are marked by a dashed and double underline, respectively. The presumed recognition site for proteolytic cleavage is underlined. The R-A-L sequence, which corresponds to the H-A-V sequence of typical cadherins is boxed. Putative Ca^{2+}-binding sites are shaded. Horizontal arrows under the amino acid sequence show beginning of each domain. Horizontal arrows over nucleotide sequence indicate regions of isolated clones. Vertical arrows indicate potential N-glycosylation sites. * indicates stop codon. (GenBank accession number M76482).

Figure 8. Multiple amino acid sequence alignment of human PVA (pv), human DGI (dg), and human P-cadherin (pc). DGI (Nilles et al., 1991) and P-cadherin (Shimoyama et al., 1989) are from published

sequences. The sequences for each molecule start with the proteolytic cleavage recognition site then are divided into domains, as explained in the text. (The amino acid numbers for these domains are found in Figure 7). Amino acid residues of PVA that are conserved in DGI or P-cadherin are shaded. Solid lines overlie putative Ca^{2+}-binding sites. Vertical arrows indicate potential N-glycosylation sites shared by PVA and DGI. *'s indicate R-A-L sequence of PVA and DGI that corresponds to P-cadherin's H-A-V sequence. Cysteine residues of PVA that are conserved in DGI or P-cadherin are shown in reverse highlight. The +'s indicate the repetitive N-V/Y-X-V-T-E domains shared by PVA and DGI. The identity and similarity of DGI and P-cadherin to PVA are shown for each domain to the right of the sequences. (NS indicates that similarity is not significant).

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a glycoprotein, phemphigus vulgaris antigen (PVA), that is involved in the disease pemphigus vulgaris (PV), an autoimmune disease of skin and mucous membranes in which autoantibodies against the surface of keratinocyte cells cause loss of cell-to-cell adhesion and blister formation. The autoantibodies are specific to PVA, which has been characterized as a 130-kD glycoprotein linked by disulfide bonds to plakoglobin.

In one embodiment, this invention relates to DNA sequences (including cDNA sequences) that encode PVA. The invention further relates to DNA sequences that encode the entire amino acid sequence given in Figure 7 (the specific DNA sequence given in Figure 7 being only one example), or any portion

comprising at least 12 base pairs thereof. DNA sequences to which the invention relates also include those encoding proteins (or polypeptides) having substantially the same autoantibody binding characteristics of PVA (for example, allelic forms of the amino acid sequence of Figure 7).

That the cDNA cloned encodes the 130-kD PVA is supported by several observations, as demonstrated by the examples below: 1) PV sera, but not normal or disease control sera, bind the fusion protein derived from the initially isolated clone (MJ315) 2) Epitope selection of antibodies from PV sera by this clone resulted in IgG that stained monkey esophagus by immunofluorescence in the same cell surface pattern as do PV sera, and that bound the 130-kD PVA.

3) Rabbit sera raised against the MJ315 fusion protein also showed PV-like immunofluorescence and bound the 130-kD PVA by immunoblotting 4) Extension clones E12 and E33, as well as initial clone MJ315, hybridized to the same size mRNAs, which were large enough to encode the PVA 5) The tissue specificity of mRNA expression for PVA is consistent with the known tissue distribution of PVA (i.e. stratified squamous epithelia only). 6) The initial and extension clones contain one long continuous open reading frame encoding a protein of approximately the correct molecular weight and isoelectric point.

In another embodiment, the present invention also relates to proteins (or polypeptides) having an amino acid sequence corresponding to any portion that is at least 4 amino acids of the protein depicted in Figure 7 (or allelic variations thereof). As an example, the protein (or polypeptide) can have an amino acid sequence

corresponding to an epitope of the sequence of Figure 7 (or allelic variation thereof). Furthermore, the protein can be used as an antigen, in protocols known in the art, to produce antibodies thereto, both monoclonal and polyclonal.

In another embodiment, the present invention relates to a recombinant DNA molecule that includes a vector and a DNA sequence as described above (advantageously, a DNA sequence encoding the protein shown in Figure 7 or a protein having the autoantibody binding characteristics of that protein). The vector can take the form of a virus, a plasmid, or eukaryotic expression vector (for example, lambda gTII, pUEX, bacillovirus vectors and pcDNAIneo expression vectors). The DNA sequence can be present in the vector operably linked to regulatory elements, including, for example, a promoter. The recombinant molecule can be suitable for transforming procaryotic or transfecting eukaryotic cells, advantageously, mammalian cells or insect cells. For instance, pUEX plasmids are suitable for transforming bacterial cells, and pcDNAIneo vector is suitable for eukaryotic transfection.

In a further embodiment, the present invention relates to host cells stably transformed or transfected with the above-described recombinant constructs. The host cell can be prokaryotic (for example, bacterial), lower eukaryotic (for example, yeast or insect) or higher eukaryotic (for example, all mammals, including but not limited to mouse and human). For instance, transient or stable transfections can be accomplished into chinese hamster ovary cells (CHO) or COS-7 cells. Transformation or transfection can be accomplished

using protocols and materials well known in the art. The transformed or transfected host cells can be used as a source of the DNA sequences described above (which sequence constitutes part of the recombinant construct). When the recombinant molecule takes the form of an expression system, the transformed or transfected cells can be used as a source for the above-described PVA protein.

In a further embodiment, the present invention relates to a method of producing PVA which includes culturing the above-described host cells, under conditions such that the DNA fragment is expressed and PVA is produced thereby. The PVA can then be isolated using methodology well known in the art. The PVA produced can be used in the diagnosis or treatment of persons having PV.

In another embodiment, the present invention relates to antibodies specific for the above-described proteins (or polypeptides). For instance, an antibody can be raised against a peptide having the amino acid sequence of Figure 7, or against a portion thereof of at least 4 amino acids in length. Persons skilled in the art using standard methodology can raise monoclonal and polyclonal antibodies to the protein (or polypeptide), or a unique portion thereof.

In a further embodiment, the present invention relates to a method of detecting the presence of PVA or antibodies against PVA in a sample. Using standard methodology well known in the art, a diagnostic assay can be constructed by coating on a surface (i.e. a solid support) for example, a microtitration plate or a membrane (e.g. nitrocellulose membrane), all or a unique portion of the synthetic PVA protein described above, and

contacting it with the serum of a person suspected of having PV. The presence of a resulting complex formed between the PVA and antibodies specific therefor in the serum can be detected by any of the known methods common in the art, such as fluorescent antibody spectroscopy or colorimetry. This method of detection can be used, for example, for the diagnosis of PV.

In another embodiment, the present invention relates to a diagnostic kit which contains recombinantly produced PVA and ancillary reagents that are well known in the art and that are suitable for use in detecting the presence of antibodies to PVA in serum or a tissue sample. Tissue samples contemplated can be monkey and human, or other mammals such as dog.

In a further embodiment, the present invention relates to a therapeutic method for the treatment of PV disease. Plasmapheresis can be conducted on an individual having PV. Before reinfusion of the blood back into the individual, persons skilled in the art using standard methodology can contact the individual's blood with the synthetic PVA described above. The blood can then be reinfused into the individual.

EXAMPLES

The following technical protocols and materials are used in the examples that follow:

Human Sera

Sera from patients with clinically and histologically typical PV showed characteristic cell surface immunofluorescence on monkey esophagus and immunoprecipitated the 130-kD PVA (Stanley, 1989). Control sera were obtained from patients with clinically and histologically typical pemphigus foliaceous and bullous pemphigoid. These sera also showed characteristic immunofluorescence findings (Stanley, 1989). Finally, normal human sera were also used as controls.

Cell Culture

NHEK (Clonetics) were cultured in keratinocyte growth medium (Clonetics) which has a Ca^{2+} concentration of 0.15 mM. In some experiments the Ca^{2+} concentration was raised to 2.55 mM for 24 hr before RNA extraction and for 48 hr before indirect immunofluorescence. To determine the effects of N-glycosylation on the immunoreactivity of PVA, human foreskin epidermal cells were cultured on 3T3 cells as previously described (Rheinwald and Green, 1975; Fuchs and Green, 1981; Stanley et al., 1984), either with or without 2.5 μg/ml tunicamycin (Sigma), which was added for 1 hr before the addition of ^{14}C-amino acids. Cells were radiolabeled overnight, then extracted for immunoprecipitation, as previously described (Stanley et al., 1984).

Immunofluorescence

Indirect immunofluorescence with PV sera or rabbit antisera was performed on monkey esophagus, the standard substrate to detect PVA with patients' sera, or on cultured NHEK as previously described (Sabolinski et al., 1987; Stanley et al., 1981, 1982).

Immunoblotting and Affinity Purification of PV IgG

Proteins from cultured NHEK were extracted with sodium dodecyl sulfate (SDS) sample buffer with reduction, separated by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to nitrocellulose membranes (Hashimoto et al., 1990; Towbin et al., 1979). Immunoblotting was performed with human sera or rabbit antisera and alkaline phosphatase labeled goat anti-human or anti-rabbit IgG (Stanley et al., 1984; Amagai et al., 1990). For affinity purification of PV IgG, horizontal strips of nitrocellulose containing the 130-kD PVA were cut out, incubated wtih PV serum, washed, then bound antibodies were eluted with acid glycine buffer, neutralized, dialyzed against phosphate buffered saline, and concentrated as described (Mueller et al., 1989).

Construction and Screening of cDNA Library

Total RNA was extracted from cultured NHEK with LiCl buffer (Amagai et al., 1990) and poly(A)$^+$ RNA was purified twice with an oligo(dT) column (Stratagene). cDNA was synthesized with random primers and the reverse transcriptase Superscript (Gibco-BRL) by the basic method of Gubler and Hoffman (Gubler and Hoffman, 1983). The cDNA was

ligated with EcoRI/NotI adaptors (Invitrogen) in order to insert into the EcoRI site of λgt11 (Young and Davis, 1983) or λZapII (Stratagene) then packaged (Stratagene). Approximately 10^6 independent recombinants were screened by immunostaining (Amagai et al., 1990) using affinity-purified PV antibodies. Positive clones were plaque-purified through several rounds of re-screening.

For extension cloning, the cDNA library was screened at high stringency by hybridization with MJ315 labeled with ^{32}P by random primer labeling (Maniatis et al., 1982). From approximately 10^6 recombinant clones, E12 and E33 were isolated, and plaque purified.

The cDNA inserts from these purified plaques were subcloned into the plasmid vector pGEM (Promega) or pBluescript (Stratagene) for further characterization.

Epitope Selection

Plaque lifts of nitrocellulose-bound fusion protein produced by MJ315 in λgt11 were used to affinity purify antibodies from the PV serum as described previously (Stanley et al., 1988).

Rabbit Immunization with MJ315 Fusion Protein

The MJ315 cDNA insert was excised from its pGEM plasmid vector by amplification with polymerase chain reaction (PCR) with primers that annealed to both ends and that included either a BamHI or PstI site, so that the insert could be directionally subcloned, in frame, into the BamHI-PstI site of the expression plasmid vector pUEX 1 (Amersham). The crude β-galactosidase fusion protein produced by

pUEX was isolated as previously described for fusion proteins produced in pEX (Tanaka et al., 1990). The precipitated fusion protein was then partially purified by washing first with 0.5% Triton X-100 in 150 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl pH 7.5, then with 2 M urea in 100 mM Tris-HCl pH 8. Rabbits were immunized subcutaneously with approximately 500 μg of this partially purified fusion protein mixed with complete (first immunization) or incomplete Freund's adjuvant, every 2 weeks for a total of 3 injections.

Northern and Southern Blot Analysis

Poly(A)$^+$ RNA for Northern analysis was isolated from cultured NHEK and normal human fibroblasts as described above. Poly (A)$^+$ RNA was also extracted from monkey esophagus and tongue (Invitrogen Fast Track System). Poly(A)$^+$ RNA from human and other monkey tissues were also used (Clontech). Approximately 2 μg of each RNA was resolved in a 1% agarose/formaldehyde gel, transferred by blotting to a nylon membrane (Genescreen Plus, Dupont), and hybridized at 42° in 50% formamide with ^{32}P-labeled MJ315 cDNA (Amagai et al., 1991). Duplicate lanes of RNA, run in parallel, were used for ^{32}P-labeled β-actin cDNA hybridization.

For Southern analysis, human placental DNA (Oncor) was digested with EcoRI, HindIII, BamHI, PstI, and BglII and electrophoresed in a 0.7% agarose gel, then transferred by vacuum blotting to a nylon membrane and hybridized to ^{32}P-labeled MJ315, as described (Amagai et al., 1991).

Sequenc Analysis

Double stranded cDNA in pGEM or pBluescript was sequenced in both directions by the dideoxy chain termination method with Sequenase (United States Biochemical Corp.). Oligonucleotides, corresponding to vector or previously-determined sequence, were synthesized to use as primers.

Homology searches against GenPep (Release 64.3), PIR-Protein (Release 28), SwissProt (Release 18) with FASTA, sequence comparison with GAP, and multiple sequence alignment with PILEUP were done with the University of Wisconsin Genetics Computer Group software on a VAX (Devereux et al., 1984). PC/Gene software (Intelligenetics) was used to determine: a) statistical significance of amino acid identities and similarities between corresponding regions of PVA with DGI and P-cadherin, as well as between extracellular domains of PVA (PCOMPARE), and b) transmembrane regions and signal peptides.

Example I

Further Characterization of PVA and Affinity Purification of PV IgG to Optimize Cloning

To determine if we could screen prokaryotic expression vectors with PV sera, we wanted to be sure that the antigenic moiety of the PVA glycoprotein did not reside in, or depend on, N-linked complex carbohydrates. We therefore cultured NHEK in the presence or absence of tunicamycin, which blocks N-glycosylation, and immunoprecipitated extracts of these cells with PV sera. From cells cultured with or without tunicamycin, PV sera specifically precipitated approximately 115-kD and 130-kd molecules, respectively (Fig. 1). Therefore, we conclude that N-linked sugars add about 15 kD to

the molecular weight of PVA and the antigenic specificity of PV antibodies does not depend on N-glycosylation.

We, therefore, used sera from patients with PV to screen a λgt11 expression library of cDNA obtained from normal human epidermal keratinocytes (NHEK) cultured in 0.15 mM Ca^{2+} medium. Preliminary immunoprecipitation studies showed that these cells synthesize PVA (data not shown). Over 200 candidate clones were identified by various PV sera, but none of these could be confirmed to be correct, because although the protein products of these clones bound the PV screening serum, either they also bound other normal or disease control sera or they did not bind any other PV sera. Therefore we tried to optimize our cloning procedure to yield more λ colonies encoding PVA and to use antibodies which would result in fewer false positive clones.

Data with mouse keratinocytes suggest that PVA synthesis is increased by raising the Ca^{2+} concentration of the growth medium (Stanley and Yuspa, 1983). We found similar results, as determined by immunofluorescence and immunoprecipitation, with these human cells, and therefore used keratinocytes grown in 2.55 mM Ca^{2+} for 24 hr for constructing a λgt11 cDNA library.

Finally, to decrease the detection of false positive clones by whole sera from PV patients, we affinity-purified a PV serum on immunoblots of the 130-kD PVA. This affinity-purified IgG stained the cell surface of monkey esophagus epithelial cells (Fig 2A) in the same pattern as do PV sera and bound only the 130-kD PVA

on immunoblots, whereas the whole sera bound additional bands (Fig 3, lanes 1,2).

Example II

Isolation of cDNA Clones for PVA

The affinity-purified anti-PVA antibodies were used to screen a λgt11 library constructed from poly(A)$^+$ RNA extracted from NHEK cultured in 2.55 mM Ca^{2+}. Of 10^6 recombinant clones, one (cDNA insert designated MJ315), which strongly bound the affinity-purified PVA antibodies, but not normal human sera, was characterized further. The 0.7 kb MJ315 cDNA insert was sequenced and found to contain one continuous open reading frame (Fig 7). The cDNA was then subcloned, in frame, into the expression plasmid pUEX 1. The MJ315-β-galactosidase fusion protein was produced and tested by immunoblotting with PV sera, as well as pemphigus foliaceous and bullous pemphigoid disease control sera and normal sera. (Pemphigus foliaceous and bullous pemphigoid are autoantibody-mediated blistering skin diseases, whose autoantigens are distinct from PVA (Stanley, 1989)). This fusion protein was recognized by 7 out of 23 PV sera, but not by any of 19 pemphigus foliaceous, 14 bullous pemphigoid or 10 normal sera (Fig 4). We conclude that MJ315 encodes epitopes that specifically bind PV antibodies. However, not all PV sera are capable of recognizing the limited epitopes expressed on immunoblots by the MJ315 fusior protein.

To confirm that the antibodies which bind to the protein encoded by MJ315 also bind to the cell surface of stratified squamous epithelial cells and the 130-kD PVA, we used epitope selection to

affinity-purify the IgG from PV serum incubated on the λ clone of MJ315. This affinity-purified IgG, but not IgG affinity purified from PV serum on irrelevant clones, showed cell surface immunofluorescence on monkey esophagus epithelium, indistinguishable from that of PV sera (Fig 2B,C), and bound the 130-kD PVA on immunoblots (Fig 3, lanes 3,4).

Finally, we confirmed that MJ315 encodes PVA by immunizing rabbits with the MJ315 fusion protein made in pUEX 1. These rabbit antibodies stained monkey esophagus in the same cell surface pattern as PV sera (Fig 2D), and bound the 130-kD PVA by immunoblotting (data not shown).

In order to isolate cDNA with the entire coding sequence for PVA, we screened λgt11 and λZAPII keratinocyte cDNA libraries with ^{32}P-labeled MJ315 and the 5' 200 bp of MJ315. We isolated two extended, overlapping clones (cDNA inserts designated E12 and E33), which contained the entire coding region of PVA (Fig 7).

Example III

Northern and Southern Analysis

Northern analysis of mRNA extracted from cultured NHEK with MJ315, E12, or E33 each indicated a major 6 kb and minor 4 and 3.5 kb bands (Fig 5). The size of the RNA is large enough to encode a 115 kD protein. Because the detection of PVA by immunofluorescence is limited to stratified squamous epithelia (Beutner et al., 1968), we determined whether mRNA for PVA was expressed in cells and tissues of stratified squamous epithelia (keratinocytes, esophagus, tongue) compared to other cells and tissues (fibroblasts, brain, heart, lung,

liver, kidney, placenta, and small intestine). mRNA for PVA was detected only in stratified squamous epithelia (Fig. 5).

Southern analysis of human genomic DNA digested with EcoRI, HindIII, and PstI and hybridized with MJ315 showed a single band, which suggests that PVA is encoded by a single gene (Fig. 6).

Example IV

Analysis of the Deduced Amino Acid Sequence of PVA and Comparison with Cadherin Family

DNA sequencing of the overlapping PVA cDNA clones indicated a total 3,336 bp cDNA with a 2,997 bp open reading frame (Fig 7). There are two tandem ATG potential translation initiation codons after an upstream in-frame stop codon. Either could be the initiation codon, however the bases surrounding the second ATG codon are more consistent with a consensus initiation sequence (Kozak, 1987). Either one of the potential initiation methionines starts what is predicted to be a hydrophobic signal sequence. Hydrophobicity plots also identified a putative transmembrane region. There is a stop codon at bases 3081-3, and two more in frame stop codons within 10 codons after it. There is a 256 bp, incomplete, 3' non-coding region.

Comparison of the PVA amino acid sequence to protein databases indicated significant homology only to members of the cadherin family, most markedly to DGI. The overall similarity/identity of PVA to human (Wheeler et al., 1991; Nilles et al., 1991) and bovine DGI (Koch et al., 1990; Goodwin et al., 1990) was 64%/46% and 65%/48%, respectively. There was also significant similarity/identity to

the typical cadherins: human P-cadherin (Shimoyama et al., 1989) 47%/25%, mouse P-cadherin (Nose et al., 1987) 48%/25%, human N-cadherin (Walsh et al., 1990) 47%/29%, chick N-cadherin (Hatta et al., 1988) 48%/28%. This similarity of PVA to the typical cadherins was about the same as to the recently cloned bovine desmocollins I/II (Collins et al., 1991; Mechanic et al., 1991), also of the cadherin family: 47%/28%. We conclude that PVA is a member of the cadherin family, and that it is more closely related to DGI than to the typical cadherins. Since this similarity is the same across species lines, it suggests that the conserved areas may subserve important functions.

These conserved areas are demonstrated in Fig 8, in which PVA is compared with human DGI and human P-cadherin, a representative, typical cadherin. By homology with cadherins, it can be deduced that the mature PVA protein is probably cleaved from a precursor protein after a conserved sequence of basic amino acids with the sequence R-R-X-K-R (Shirayoshi et al., 1986; Gallin et al., 1987; Goodwin et al., 1990; Koch et al., 1990; Collins et al., 1991; Mechanic et al., 1991; Ozawa and Kemler, 1990) (Figs. 7,8). This cleavage would result in a mature PVA unglycosylated peptide of 950 amino acids with molecular weight 102 kD and pI 4.5. This is in fairly good agreement with the estimated molecular weight of PVA extracted from cells cultured with tunicamycin (Fig. 1) and with a pI for PVA estimated at 5 (Eyre and Stanley, 1988).

The extracellular region of PVA, by homology to typical cadherins (Hatta et al., 1988; Shimoyama et al., 1989), can be divided into 5 domains of about equal size (Figs 7,8), EC1 to EC5,

which, except for EC5, have homology with each other. As for typical cadherins, the homology is greatest among EC1, EC2 and EC3, the most amino-terminal domains (Ringwald et al., 1987; Takeichi, 1991). Similarly, the extracellular regions of DGI and desmocollin have been divided into 5 domains, only the first four of which in DGI are homologous to typical cadherins (Koch et al., 1990; Nilles et al., 1991; Collins et al., 1991; Mechanic et al., 1991). All five extracellular regions of PVA show significant homology to corresponding domains in P-cadherin. However, in domains EC1, EC2, and EC3 the homology of PVA to DGI is much greater than to P-cadherin. Unlike DGI, which has a shortened EC5 region, the EC5 region of PVA is similar in size to that of P-cadherin. The highly conserved sequence H-A-V of typical cadherins (Takeichi, 1990), thought to be important in cell adhesion (Blaschuk et al., 1990; Nose et al., 1990), is represented in PVA and DGI by the conservatively substituted sequence R-A-L (Figs 7,8) (Koch et al., 1990; Goodwin et al., 1990; Wheeler et al., 1991).

Other conserved sequences in the extracellular domains of PVA and cadherins with potential function include putative Ca^{2+}-binding motifs (D-X-N-D-N and A/V-X-D-X-D) (Figs 7,8) (Ringwald et al., 1987; Ozawa et al., 1990). In addition, 2 of 4 potential N-glycosylation sites in PVA are conserved in equivalent positions in DGI (Fig 8).

The cytoplasmic domain of PVA (360 amino acids) is substantially longer than that of typical cadherins (approximately 160 residues) but shorter than that of DGI (480 residues). Unlike typical cadherins, which do not contain cysteines in the

cytoplasmic domain, PVA and DGI each have 5 cysteines in equivalent positions (Fig 8). By homology with DGI the cytoplasmic region of PVA can be divided into 4 subdomains. (Koch et al., 1990; Nilles et al., 1991) (Figs 7,8). PVA is missing a fifth glycine rich C-terminal cytoplasmic domain found in DGI (Koch et al., 1990; Nilles et al., 1991). The IA ("intracellular anchor") region of PVA is homologous to that of DGI, but unlike that of typical cadherins, which have basic amino acids just inside the membrane. The C1 region of PVA is similar to DGI and typical cadherins, but as with EC1-EC3, the similarity is much greater with DGI. Finally, the C3 region of PVA has two of the five N-V-X-V-T-E repeats that are found in DGI (Nilles et al., 1991).

Since autoantibodies in PV patients have been shown to mediate loss of epidermal cell adhesion with resultant blister formation, it seems particularly relevant that analysis of the deduced amino acid sequence for PVA indicated homology to the cadherin family of cell adhesion molecules. Cadherins are Ca^{2+}-dependent cell-cell adhesion molecules that mediate homophilic binding (Takeichi, 1991, 1990). These molecules are thought to be important in establishing and maintaining epithelial and neural tissue integrity. The typical cadherins, which were the first defined, are now well characterized at a molecular level and include E-cadherin (Ringwald et al., 1987; Nagafuchi et al., 1987), N-cadherin (Hatta et al., 1988; Miyatani et al., 1989; Walsh et al., 1990), P-cadherin (Nose et al., 1987; Shimoyama et al., 1989), and L-CAM (Gallin et al., 1987). These form a closely related family of molecules with very well conserved

extracellular and cytoplasmic domains (Takeichi, 1991, 1990). Studies utilizing monoclonal antibody inhibition of homophilic binding, site-directed mutagenesis, and production of chimeric cadherin molecules have shown that the amino terminal 113 residues are important for determining binding function and specificity of binding (Nose et al., 1990; Takeichi, 1990). Calcium binding to the first Ca^{2+}-binding motif in EC2 has also been shown to be critical for preserving adhesive function (Ozawa et al., 1990). In addition, the very well conserved cytoplasmic portion of cadherins is also crucial for homophilic binding (Nagafuchi and Takeichi, 1988) as well as for binding catenins and CAP 102, cadherin-associated proteins that may anchor cadherins to the actin cytoskeleton (Ozawa et al., 1989, 1990; Nagafuchi et al., 1991).

Recently DGI and desmocollins, transmembrane glycoproteins that extend into the core of desmosomes (Gorbsky and Steinberg, 1981; Mueller and Franke, 1983; Cowin et al., 1984; Miller et al., 1987; Steinberg et al., 1987), have been cloned (Koch et al., 1990; Goodwin et al., 1990; Wheeler et al., 1991; Nilles et al., 1991; Collins et al., 1991; Mechanic et al., 1991). Both were found to be related to typical cadherins in their extracellular domains and part of their cytoplasmic portions. Desmocollins are no more similar to DGI than to typical cadherins. Although PVA may also be found in desmosomes, it is not necessarily concentrated in these junctions, but may be found uniformly on the keratinocyte cell surface (Wolff and Schreiner, 1971; Jones et al., 1986b).

PVA shows significant homology to all cadherins, but most markedly to DGI. This homology

extends across species, suggesting that the conserved regions may be functionally important. Like all other members of the cadherin family, PVA has a putative signal sequence and a well conserved sequence of basic amino acids that presumably serve as a signal for cleavage to a mature protein. PVA, like typical cadherins, can be divided into five extracellular domains, of which EC1 to EC4 show variable homology to each other. Like typical cadherins, EC5 shows minimal or no significant homology to the other extracellular domains. Near the amino terminus of the mature protein, which is the area containing important sites for homophilic binding in typical cadherins, PVA shows much greater similarity to corresponding domains of DGI than to those of typical cadherins. Like DGI, PVA has an R-A-L site in EC1 that corresponds to the conserved H-A-V site in an equivalent position in typical cadherins. PVA also has several conserved putative Ca^{2+} binding domains with all cadherins as well as two conserved N-glycosylation sites with DGI. Glycosylation at the four potential extracellular N-glycosylation sites of PVA could account for the 15 kD difference in molecular weight of PVA synthesized in the presence or absence of tunicamycin. The cytoplasmic domains of PVA are also most similar to those of DGI. Most remarkably, PVA and DGI share 5 cysteines in equivalent positions, whereas typical cadherins lack cysteines. This could be significant in that PVA, like DGI, binds plakoglobin by disulfide bonds (Korman et al., 1989), whereas typical cadherins bind catenins and CAP 102 presumably by noncovalent bonds (Ozawa et al., 1989, 1990; Nagafuchi et al., 1991). These sequence comparison data indicate that DGI and PVA are both

in the cadherin family of proteins, but are more closely related to each other than to typical cadherins or to desmocollins. Thus, PVA and DGI form a subfamily of cadherins.

Like PVA, DGI is also a target antigen in another autoantibody-mediated blistering disease of the epidermis, pemphigus foliaceous (Koulu et al., 1984; Stanley et al., 1986; Eyre and Stanley, 1987). Pemphigus foliaceous is clinically and histologically distinct from PV. The blister in pemphigus foliaceous occurs more superficially within the epidermis than does the blister in PV. As in PV, pemphigus foliaceous autoantibodies have been shown to mediate loss of cell adhesion and blister formation (Hashimoto et al., 1983; Roscoe et al., 1985; Rock et al., 1990). Thus, in the two known IgG autoantibody-mediated blistering diseases of epidermis, cadherin-like molecules are the target antigens. However, from previous immunofluorescence studies as well as the Northern data presented here, expression of PVA is limited to stratified squamous epithelia, whereas DGI is present in all desmosome-containing tissues (Cowin and Garrod, 1983; Schmelz et al., 1986). Alternatively, PVA might be considered to be a tissue-specific type of desmoglein. In any case, autoantibodies from PV patients define a novel cadherin, limited to stratified squamous epithelia, and a target of an autoimmune disease that results in blisters in these tissues. These findings suggest that a novel cadherin is important in the normal structure and maintenance of adult epidermis and can be a target of disease.

Although various pathophysiologic mechanisms of blister formation have been proposed

in PV (Hashimoto et al., 1983; Morioka et al., 1987; Sams and Gammon, 1982), it must now be considered that autoantibodies in these patients may interfere directly with the function of PVA as an adhesion molecule. Cloning of PVA, and the fact that there is a good animal model for inducing the disease with passive transfer of IgG (Anhalt et al., 1982), now makes it feasible to determine whether antibodies (either from patients or raised in animals) directed against certain epitopes are associated with increased severity of disease in humans and/or are capable of inducing disease in animals. These types of studies should lead to a more detailed understanding of the role for this novel epithelial cadherin in normal epidermis and in disease.

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CLAIMS:

1. A DNA fragment that encodes pemphigus vulgaris antigen.

2. The DNA fragment according to claim 1, wherein said fragment has the sequence:

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                10         20         30         40         50         6(
                |          |          |          |          |
  1 TTTTCTTAGA CATTAACTGC AGACGGCTGG CAGGATAGAA GCAGCGGCTC ACTTGGACT
    AAAAGAATCT GTAATTGACG TCTGCCGACC GTCCTATCTT CGTCGCCGAG TGAACCTGA

 61 TTTCACCAGG GAAATCAGAG ACAATGATGG GGCTCTTCCC CAGAACTACA GGGGCTCTG
    AAAGTGGTCC CTTTAGTCTC TGTTACTACC CCGAGAAGGG GTCTTGATGT CCCCGAGAC

121 CCATCTTCGT GGTGGTCATA TTGGTTCATG GAGAATTGCG AATAGAGACT AAAGGTCAA
    GGTAGAAGCA CCACCAGTAT AACCAAGTAC CTCTTAACGC TTATCTCTGA TTTCCAGTT

181 ATGATGAAGA AGAGATGACT ATGCAACAAG CTAAAAGAAG GCAAAAACGT GAATGGGTG
    TACTACTTCT TCTCTACTGA TACGTTGTTC GATTTTCTTC CGTTTTTGCA CTTACCCAC

241 AATTTGCCAA ACCCTGCAGA GAAGGAGAAG ATAACTCAAA AAGAAACCCA ATTGCCAAGA
    TTAAACGGTT TGGGACGTCT CTTCCTCTTC TATTGAGTTT TTCTTTGGGT TAACGGTTCT

301 TTACTTCAGA TTACCAAGCA ACCCAGAAAA TCACCTACCG AATCTCTGGA GTGGGAATCG
    AATGAAGTCT AATGGTTCGT TGGGTCTTTT AGTGGATGGC TTAGAGACCT CACCCTTAGC

361 ATCAGCCGCC TTTTGGAATC TTTGTTGTTG ACAAAAACAC TGGAGATATT AACATAACAG
    TAGTCGGCGG AAAACCTTAG AAACAACAAC TGTTTTTGTG ACCTCTATAA TTGTATTGTC

421 CTATAGTCGA CCGGGAGGAA ACTCCAAGCT TCCTGATCAC ATGTCGGGCT CTAAATGCCC
    GATATCAGCT GGCCCTCCTT TGAGGTTCGA AGGACTAGTG TACAGCCCGA GATTTACGGG

481 AAGGACTAGA TGTAGAGAAA CCACTTATAC TAACGGTTAA AATTTTGGAT ATTAATGATA
    TTCCTGATCT ACATCTCTTT GGTGAATATG ATTGCCAATT TTAAAACCTA TAATTACTAT

541 ATCCTCCAGT ATTTTCACAA CAAATTTTCA TGGGTGAAAT TGAAGAAAAT AGTGCCTCAA
    TAGGAGGTCA TAAAAGTGTT GTTTAAAAGT ACCCACTTTA ACTTCTTTTA TCACGGAGTT

601 ACTCACTGGT GATGATACTA AATGCCACAG ATCCAGATGA ACCAAACCAC TTGAATTCTA
    TGAGTGACCA CTACTATGAT TTACGGTGTC TAGGTCTACT TGGTTTGGTG AACTTAAGAT

661 AAATTGCCTT CAAAATTGTC TCTCAGGAAC CAGCAGGCAC ACCCATGTTC CTCCTAAGCA
    TTTAACGGAA GTTTTAACAG AGAGTCCTTG GTCGTCCGTG TGGGTACAAG GAGGATTCGT

721 GAAACACTGG GGAAGTCCGT ACTTTGACCA ATTCTCTTGA CCGAGAGCAA TCTAGCAGCT
    CTTTGTGACC CCTTCAGGCA TGAAACTGGT TAAGAGAACT GGCTCTCGTT CGATCGTCGA

781 ATCGTCTGGT TGTGAGTGGT GCAGACAAAG ATGGAGAAGG ACTATCAACT CAATGTGAAT
    TAGCAGACCA ACACTCACCA CGTCTGTTTC TACCTCTTCC TGATAGTTGA GTTACACTTA

841 GTAATATTAA AGTGAAAGAT GTCAACGATA ACTTCCCAAT GTTTAGAGAC TCTCAGTATT
    CATTATAATT TCACTTTCTA CAGTTGCTAT TGAAGGGTTA CAAATCTCTG AGAGTCATAA

901 CAGCACGTAT TGAAGAAAAT ATTTTAAGTT CTGAATTACT TCGATTTCAA GTAACAGATT
    GTCGTGCATA ACTTCTTTTA TAAAATTCAA GACTTAATGA AGCTAAAGTT CATTGTCTAA
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 961 TGGATGAAGA GTACACAGAT AATTGGCTTG CAGTATATTT CTTTACCTCT GGGAATGAAG
     ACCTACTTCT CATGTGTCTA TTAACCGAAC GTCATATAAA GAAATGGAGA CCCTTACTTC

1021 GAAATTGGTT TGAAATACAA ACTGATCCTA GAACTAATGA AGGCATCCTG AAAGTGGTGA
     CTTTAACCAA ACTTTATGTT TGACTAGGAT CTTGATTACT TCCGTAGGAC TTTCACCACT

1081 AGGCTCTAGA TTATGAACAA CTACAAAGCG TGAAACTTAG TATTGCTGTC AAAAACAAAG
     TCCGAGATCT AATACTTGTT GATGTTTCGC ACTTTGAATC ATAACGACAG TTTTTGTTTC

1141 CTGAATTTCA CCAATCAGTT ATCTCTCGAT ACCGAGTTCA GTCAACCCCA GTCACAATTC
     GACTTAAAGT GGTTAGTCAA TAGAGAGCTA TGGCTCAAGT CAGTTGGGGT CAGTGTTAAG

1201 AGGTAATAAA TGTAAGAGAA GGAATTGCAT TCCGTCCTGC TTCCAAGACA TTTACTGTGC
     TCCATTATTT ACATTCTCTT CCTTAACGTA AGGCAGGACG AAGGTTCTGT AAATGACACG

1261 AAAAAGGCAT AAGTAGCAAA AAATTGGTGG ATTATATCCT GGGAACATAT CAAGCCATCG
     TTTTTCCGTA TTCATCGTTT TTTAACCACC TAATATAGGA CCCTTGTATA GTTCGGTAGC

1321 ATGAGGACAC TAACAAAGCT GCCTCAAATG TCAAATATGT CATGGGACGT AACGATGGTG
     TACTCCTGTG ATTGTTTCGA CGGAGTTTAC AGTTTATACA GTACCCTGCA TTGCTACCAC

1381 GATACCTAAT GATTGATTCA AAAACTGCTG AAATCAAATT TGTCAAAAAT ATGAACCGAG
     CTATGGATTA CTAACTAAGT TTTTGACGAC TTTAGTTTAA ACAGTTTTTA TACTTGGCTC

1441 ATTCTACTTT CATAGTTAAC AAAACAATCA CAGCTGAGGT TCTGGCCATA GATGAATACA
     TAAGATGAAA GTATCAATTG TTTTGTTAGT GTCGACTCCA AGACCGGTAT CTACTTATGT

1501 CGGGTAAAAC TTCTACAGGC ACGGTATATG TTAGAGTACC CGATTTCAAT GACAATTGTC
     GCCCATTTTG AAGATGTCCG TGCCATATAC AATCTCATGG GCTAAAGTTA CTGTTAACAG

1561 CAACAGCTGT CCTCGAAAAA GATGCAGTTT GCAGTTCTTC ACCTTCCGTG GTTGTCTCCG
     GTTGTCGACA GGAGCTTTTT CTACGTCAAA CGTCAAGAAG TGGAAGGCAC CAACAGAGGC

1621 CTAGAACACT GAATAATAGA TACACTGGCC CCTATACATT TGCACTGGAA GATCAACCTG
     GATCTTGTGA CTTATTATCT ATGTGACCGG GGATATGTAA ACGTGACCTT CTAGTTGGAC

1681 TAAAGTTGCC TGCCGTATGG AGTATCACAA CCCTCAATGC TACCTCGGCC CTCCTCAGAG
     ATTTCAACGG ACGGCATACC TCATAGTGTT GGGAGTTACG ATGGAGCCGG GAGGAGTCTC

1741 CCCAGGAACA GATACCTCCT GGAGTATACC ACATCTCCCT GGTACTTACA GACAGTCAGA
     GGGTCCTTGT CTATGGAGGA CCTCATATGG TGTAGAGGGA CCATGAATGT CTGTCAGTCT

1801 ACAATCGGTG TGAGATGCCA CGCAGCTTGA CACTGGAAGT CTGTCAGTGT GACAACAGGG
     TGTTAGCCAC ACTCTACGGT GCGTCGAACT GTGACCTTCA GACAGTCACA CTGTTGTCCC

1861 GCATCTGTGG AACTTCTTAC CCAACCACAA GCCCTGGGAC CAGGTATGGC AGGCCGCACT
     CGTAGACACC TTGAAGAATG GGTTGGTGTT CGGGACCCTG GTCCATACCG TCCGGCGTGA

1921 CAGGGAGGCT GGGGCCTGCC GCCATCGGCC TGCTGCTCCT TGGTCTCCTG CTGCTGCTGT
     GTCCCTCCGA CCCCGGACGG CGGTAGCCGG ACGACGAGGA ACCAGAGGAC GACGACGACA

1981 TGGCCCCCCT TCTGCTGTTG ACCTGTGACT GTGGGGCAGG TTCTACTGGG GGAGTGACAG
     ACCGGGGGGA AGACGACAAC TGGACACTGA CACCCCGTCC AAGATGACCC CCTCACTGTC
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2041 GTGGTTTTAT CCCAGTTCCT GATGGCTCAG AAGGAACAAT TCATCAGTGG GGAATTGAAG
     CACCAAAATA GGGTCAAGGA CTACCGAGTC TTCCTTGTTA AGTAGTCACC CCTTAACTTC

2101 GAGCCCATCC TGAAGACAAG GAAATCACAA ATATTTGTGT GCCTCCTGTA ACAGCCAATG
     CTCGGGTAGG ACTTCTGTTC CTTTAGTGTT TATAAACACA CGGAGGACAT TGTCGGTTAC

2161 GAGCCGATTT CATGGAAAGT TCTGAAGTTT GTACAAATAC GTATGCCAGA GGCACAGCGG
     CTCGGCTAAA GTACCTTTCA AGACTTCAAA CATGTTTATG CATACGGTCT CCGTGTCGCC

2221 TGGAAGGCAC TTCAGGAATG GAAATGACCA CTAAGCTTGG AGCAGCCACT GAATCTGGAG
     ACCTTCCGTG AAGTCCTTAC CTTTACTGGT GATTCGAACC TCGTCGGTGA CTTAGACCTC

2281 GTGCTGCAGG CTTTGCAACA GGGACAGTGT CAGGAGCTGC TTCAGGATTC GGAGCAGCCA
     CACGACGTCC GAAACGTTGT CCCTGTCACA GTCCTCGACG AAGTCCTAAG CCTCGTCGGT

2341 CTGGAGTTGG CATCTGTTCC TCAGGGCAGT CTGGAACCAT GAGAACAAGG CATTCCACTG
     GACCTCAACC GTAGACAAGG AGTCCCGTCA GACCTTGGTA CTCTTGTTCC GTAAGGTGAC

2401 GAGGAACCAA TAAGGACTAC GCTGATGGGG CGATAAGCAT GAATTTTCTG GACTCCTACT
     CTCCTTGGTT ATTCCTGATG CGACTACCCC GCTATTCGTA CTTAAAAGAC CTGAGGATGA

2461 TTTCTCAGAA AGCATTTGCC TGTGCGGAGG AAGACGATGG CCAGGAAGCA AATGACTGCT
     AAAGAGTCTT TCGTAAACGG ACACGCCTCC TTCTGCTACC GGTCCTTCGT TTACTGACGA

2521 TGTTGATCTA TGATAATGAA GGCGCAGATG CCACTGGTTC TCCTGTGGGC TCCGTGGGTT
     ACAACTAGAT ACTATTACTT CCGCGTCTAC GGTGACCAAG AGGACACCCG AGGCACCCAA

2581 GTTGCAGTTT TATTGCTGAT GACCTGGATG ACAGCTTCTT GGACTCACTT GGACCCAAAT
     CAACGTCAAA ATAACGACTA CTGGACCTAC TGTCGAAGAA CCTGAGTGAA CCTGGGTTTA

2641 TTAAAAAACT TGCAGAGATA AGCCTTGGTG TTGATGGTGA AGGCAAAGAA GTTCAGCCAC
     AATTTTTTGA ACGTCTCTAT TCGGAACCAC AACTACCACT TCCGTTTCTT CAAGTCGGTG

2701 CCTCTAAAGA CAGCGGTTAT GGGATTGAAT CCTGTGGCCA TCCCATAGAA GTCCAGCAGA
     GGAGATTTCT GTCGCCAATA CCCTAACTTA GGACACCGGT AGGGTATCTT CAGGTCGTCT

2761 CAGGATTTGT TAAGTGCCAG ACTTTGTCAG GAAGTCAAGG AGCTTCTGCT TTGTCCGCCT
     GTCCTAAACA ATTCACGGTC TGAAACAGTC CTTCAGTTCC TCGAAGACGA AACAGGCGGA

2821 CTGGGTCTGT CCAGCCAGCT GTTTCCATCC CTGACCCTCT GCAGCATGGT AACTATTTAG
     GACCCAGACA GGTCGGTCGA CAAAGGTAGG GACTGGGAGA CGTCGTACCA TTGATAAATC

2881 TAACGGAGAC TTACTCGGCT TCTGGTTCCC TCGTGCAACC TTCCACTGCA GGCTTTGATC
     ATTGCCTCTG AATGAGCCGA AGACCAAGGG AGCACGTTGG AAGGTGACGT CCGAAACTAG

2941 CACTTCTCAC ACAAAATGTG ATAGTGACAG AAAGGGTGAT CTGTCCCATT TCCAGTGTTC
     GTGAAGAGTG TGTTTTACAC TATCACTGTC TTTCCCACTA GACAGGGTAA AGGTCACAAG

3001 CTGGCAACCT AGCTGGCCCA ACGCAGCTAC GAGGGTCACA TACTATGCTC TGTACAGAGG
     GACCGTTGGA TCGACCGGGT TGCGTCGATG CTCCCAGTGT ATGATACGAG ACATGTCTCC

3061 ATCCTTGCTC CCGTCTAATA TGACCAGAAT GAGCTGGAAT ACCACACTGA CCAAATCTGG
     TAGGAACGAG GGCAGATTAT ACTGGTCTTA CTCGACCTTA TGGTGTGACT GGTTTAGACC

3121 ATCTTTGGAC TAAAGTATTC AAAATAGCAT AGCAAAGCTC ACTGTATTGG GCTAATAATT
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     TAGAAACCTG ATTTCATAAG TTTTATCGTA TCGTTTCGAG TGACATAACC CGATTATTAA

3181 TGGCACTTAT TAGCTTCTCT CATAAACTGA TCACGATTAT AAATTAAATG TTTGGGTTCA
     ACCGTGAATA ATCGAAGAGA GTATTTGACT AGTGCTAATA TTTAATTTAC AAACCCAAGT

3241 TACCCCAAAA GCAATATGTT GTCACTCCTA ATTCTCAAGT ACTATTCAAA TTGTAGTAAA
     ATGGGGTTTT CGTTATACAA CAGTGAGGAT TAAGAGTTCA TGATAAGTTT AACATCATTT

3301 TCTTAAAGTT TTTCAAAACC CTAAAATCAT ATTCGC
     AGAATTTCAA AAAGTTTTGG GATTTTAGTA TAAGCG
```

3. The DNA fragment according to claim 2 wherein said DNA fragment encodes the amino acid sequence:

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                5         10        15        20        25        30
                |         |         |         |         |         |
  1 M M G L F P R T T G A L A I F V V V I L V H G E L R I E T K
 31 G Q Y D E E E M T M Q Q A K R R Q K R E W V K F A K P C R E
 61 G E D N S K R N P I A K I T S D Y Q A T Q K I T Y R I S G V
 91 G I D Q P P F G I F V V D K N T G D I N I T A I V D R E E T
121 P S F L I T C R A L N A Q G L D V E K P L I L T V K I L D I
151 N D N P P V F S Q Q I F M G E I E E N S A S N S L V M I L N
181 A T D A D E P N H L N S K I A F K I V S Q E P A G T P M F L
211 L S R N T G E V R T L T N S L D R E Q A S S Y R L V V S G A
241 D K D G E G L S T Q C E C N I K V K D V N D N F P M F R D S
271 Q Y S A R I E E N I L S S E L L R F Q V T D L D E E Y T D N
301 W L A V Y F F T S G N E G N W F E I Q T D P R T N E G I L K
331 V V K A L D Y E Q L Q S V K L S I A V K N K A E F H Q S V I
361 S R Y R V Q S T P V T I Q V I N V R E G I A F R P A S K T F
391 T V Q K G I S S K K L V D Y I L G T Y Q A I D E D T N K A A
421 S N V K Y V M G R N D G G Y L M I D S K T A E I K F V K N M
451 N R D S T F I V N K T I T A E V L A I D E Y T G K T S T G T
481 V Y V R V P D F N D N C P T A V L E K D A V C S S S P S V V
511 V S A R T L N N R Y T G P Y T F A L E D Q P V K L P A V W S
541 I T T L N A T S A L L R A Q E Q I P P G V Y H I S L V L T D
571 S Q N N R C E M P R S L T L E V C Q C D N R G I C G T S Y P
601 T T S P G T R Y G R P H S G R L G P A A I G L L L L G L L L
631 L L L A P L L L L T C D C G A G S T G G V T G G F I P V P D
661 G S E G T I H Q W G I E G A H P E D K E I T N I C V P P V T
691 A N G A D F M E S S E V C T N T Y A R G T A V E G T S G M E
721 M T T K L G A A T E S G G A A G F A T G T V S G A A S G F G
751 A A T G V G I C S S G Q S G T M R T R H S T G G T N K D Y A
781 D G A I S M N F L D S Y F S Q K A F A C A E E D D G Q E A N
811 D C L L I Y D N E G A D A T G S P V G S V G C C S F I A D D
841 L D D S F L D S L G P K F K K L A E I S L G V D G E G K E V
871 Q P P S K D S G Y G I E S C G H P I E V Q Q T G F V K C Q T
901 L S G S Q G A S A L S A S G S V Q P A V S I P D P L Q H G N
931 Y L V T E T Y S A S G S L V Q P S T A G F D P L L T Q N V I
961 V T E R V I C P I S S V P G N L A G P T Q L R G S H T M L C
991 T E D P C S R L I
```

4. A DNA fragment according to claim 3, comprising at least 12 bases of the sequence set forth therein.

5. A recombinant DNA construct comprising:

(i) a vector, and

(ii) said DNA fragment according to claim 1.

6. A recombinant DNA construct comprising:

(i) a vector, and

(ii) said DNA fragment according to claim 2.

7. The recombinant DNA construct according to claim 5, wherein said vector is a eukaryotic expression vector.

8. The recombinant DNA construct according to claim 6, wherein said vector is a eukaryotic expression vector.

9. The recombinant DNA construct according to claim 5, wherein said DNA fragment encodes the amino acid sequence:

```
                    5         10        15        20        25        30
                    |         |         |         |         |         |
  1 M M G L F P R T T G A L A I F V V V I L V H G E L R I E T K
 31 G Q Y D E E E M T M Q Q A K R R Q K R E W V K F A K P C R E
 61 G E D N S K R N P I A K I T S D Y Q A T Q K I T Y R I S G V
 91 G I D Q P P F G I F V V D K N T G D I N I T A I V D R E E T
121 P S F L I T C R A L N A Q G L D V E K P L I L T V K I L D I
151 N D N P P V F S Q Q I F M G E I E E N S A S N S L V M I L N
181 A T D A D E P N H L N S K I A F K I V S Q E P A G T P M F L
211 L S R N T G E V R T L T N S L D R E Q A S S Y R L V V S G A
241 D K D G E G L S T Q C E C N I K V [ D V N D N F P M F R D S
271 Q Y S A R I E E N I L S S E L L R F Q V T D L D E E Y T D N
301 W L A V Y F F T S G N E G N W F E [ Q T D P R T N E G I L K
331 V V K A L D Y E Q L Q S V K L S I [ V K N K A E F H Q S V I
361 S R Y R V Q S T P V T I Q V I N V [ E G I A F R P A S K T F
391 T V Q K G I S S K K L V D Y I L G T Y Q A I D E D T N K A A
421 S N V K Y V M G R N D G G Y L M I D S K T A E I K F V K N M
451 N R D S T F I V N K T I T A E V L A I D E Y T G K T S T G T
481 V Y V R V P D F N D N C P T A V L E K D A V C S S S P S V V
511 V S A R T L M N R Y T G P Y T F A L E D Q P V K L P A V W S
541 I T T L N A T S A L L R A Q [ Q I P P G V Y H I S L V L T D
571 S Q N N R C E M P R S L T L [ V C Q C D N R G I C G T S Y P
601 T T S P G T R Y G R P H S G R L G P A A I G L L L L G L L L
631 L L L A P L L L L T C D C G A G S T G G V T G G F I P V P D
661 G S E G T I [ Q W G I E G A H P E D K E I T N I C V P P V T
691 A N G A D F [ [ S S E V C T N T Y A R G T A V E G T S G M E
721 M T T K L G A A T E S G G A A G F A T G T V S G A A S G F G
751 A A T G V G I C S S G Q S G T M R T R H S T G G T N K D Y A
781 D G A I S M [ F L D S Y F S Q K A F A C A E E D D G Q E A N
811 D C L L I Y C [ E G A D A [ G S P V G S V G C C S F I A D D
841 L D D S F L C S L G P K F [ K L A E I S L G V D G E G K E V
871 Q P P S K D S G Y G I E S C G H P I E V Q Q T G F V K C Q T
901 L S G S Q G A S A L S A S G S V Q P A V S I P D P L Q H G N
931 Y L V T E T Y S A S G S L V Q P S T A G F D P L L T Q N V I
961 V T E R V I C P I S S V P G N L A G P T Q L R G S H T M L C
991 T E D P C S E L I
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10. The recombinant DNA construct according to claim 6, wherein said DNA fragment encodes the amino acid sequence:

```
                5         10        15        20        25        30
                |         |         |         |         |         |
  1 M M G L F P R T T G A L A I F V V V I L V H G E L R I E T K
 31 G Q Y D E E E M T M Q Q A K R R Q K R E W V K F A K P C R E
 61 G E D N S K R N P I A K I T S D Y Q A T Q K I T Y R I S G V
 91 G I D Q P P F G I F V V D K N T G D I M I T A I V D R E E T
121 P S F L I T C R A L N A Q G L D V E K P L I L T V K I L D I
151 N D N P P V F S Q Q I F M G E I E E N S A S N S L V M I L N
181 A T D A D E P N H L N S K I A F K I V S Q E P A G T P M F L
211 L S R N T G E V R T L T N S L D R E Q A S S Y R L V V S G A
241 D K D G E G L S T Q C E C N I K V K D V N D N F P M F R D S
271 Q Y S A R I E E N I L S S E L L R F Q V T D L D E E Y T D N
301 W L A V Y F F T S G N E G N W F E I Q T D P R T N E G I L K
331 V V K A L D Y E Q L Q S V K L S I A V K N K A E F H Q S V I
361 S R Y R V Q S T P V T I Q V I N V R E G I A F R P A S K T F
391 T V Q K G I S S K K L V D Y I L G T Y Q A I D E D T N K A A
421 S N V K Y V M G R N D G G Y L M I D S K T A E I K F V K N M
451 N R D S T F I V N K T I T A E V L A I D E Y T G K T S T G T
481 V Y V R V P D F N D N C P T A V L E K D A V C S S S P S V V
511 V S A R T L N N R Y T G P Y T F A L E D Q P V K L P A V W S
541 I T T L N A T S A L L R A Q E Q I P P G V Y H I S L V L T D
571 S Q N N R C E M P R S L T L E V C Q C D N R G I C G T S Y P
601 T T S P G T R Y G R P H S G R L G P A A I G L L L L G L L L
631 L L L A P L L L L T C D C G A G S T G G V T G G F I P V P D
661 G S E G T I H Q W G I E G A H P E D K E I T N I C V P P V T
691 A N G A D F M E S S E V C T N T Y A R G T A V E G T S G M E
721 M T T K L G A A T E S G G A A G F A T G T V S G A A S G F G
751 A A T G V G I C S S G Q S G T M R T R H S T G G T N K D Y A
781 D G A I S M N F L D S Y F S Q K A F A C A E E D D G Q E A N
811 D C L L I Y D N E G A D A T G S P V G S V G C C S F I A D D
841 L D D S F L D S L G P K F K K L A E I S L G V D G E G K E V
871 Q P P S K D S G Y G I E S C G H P I E V Q Q T G F V K C Q T
901 L S G S Q G A S A L S A S G S V Q P A V S I P D P L Q H G N
931 Y L V T E T Y S A S G S L V Q P S T A G F D P L L T Q N V I
961 V T E R V I C P I S S V P G N L A G P T Q L R G S H T M L C
991 T E D P C S R L I
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11. A host cell transformed with the recombinant DNA construct according to claim 5.

12. A host cell transformed with the recombinant DNA construct according to claim 6.

13. The host cell according to claim 11, wherein said cell is a eukaryotic cell.

14. The host cell according to claim 12, wherein said cell is a eukaryotic cell.

15. A method of producing pemphigus vulgaris antigen which comprises culturing the cell according to claim 11, under conditions such that said DNA fragment is expressed and said pemphigus vulgaris antigen is thereby produced, and isolating said pemphigus vulgaris antigen.

16. A method of producing pemphigus vulgaris antigen which comprises culturing the cell according to claim 12, under conditions such that said DNA fragment is expressed and said pemphigus vulgaris antigen is thereby produced, and isolating said pemphigus vulgaris antigen.

17. The protein or glycoprotein pemphigus vulgaris antigen expressed by the DNA fragment of claim 2 having the amino acid sequence:

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                5         10        15        20        25        30
                |         |         |         |         |         |
  1 M M G L F P R T T G A L A I F V V V I L V H G E L R I E T K
 31 G Q Y D E E E M T M Q Q A K R R Q K R E W V K F A K P C R E
 61 G E D N S K R N P I A K I T S D Y Q A T Q K I T Y R I S G V
 91 G I D Q P P F G I F V V D K N T G D I N I T A I V D R E E T
121 P S F L I T C R A L N A Q G L D V E K P L I L T V K I L D I
151 N D N P P V F S Q Q I F M G E I E E N S A S N S L V M I L N
181 A T D A D E P N H L N S K I A F K I V S Q E P A G T P M F L
211 L S R N T G E V R T L T N S L D R E Q A S S Y R L V V S G A
241 D K D G E G L S T Q C E C N I K V K D V N D N F P M F R D S
271 Q Y S A R I E E N I L S S E L L R F Q V T D L D E E Y T D N
301 W L A V Y F F T S G N E G N W F E I Q T D P R T N E G I L K
331 V V K A L D Y E Q L Q S V K L S I A V K N K A E F H Q S V I
361 S R Y R V Q S T P V T I Q V I N V R E G I A F R P A S K T F
391 T V Q K G I S S K K L V D Y I L G T Y Q A I D E D T N K A A
421 S N V K Y V M G R N D G G Y L M I D S K T A E I K F V K N M
451 N R D S T F I V N K T I T A E V L A I D E Y T G K T S T G T
481 V Y V R V P D F N D N C P T A V L E K D A V C S S S P S V V
511 V S A R T L N N R Y T G P Y T F A L E D Q P V K L P A V W S
541 I T T L N A T S A L L R A Q E Q I P P G V Y H I S L V L T D
571 S Q N N R C E M P R S L T L E V C Q C D N R G I C G T S Y P
601 T T S P G T R Y G R P H S G R L G P A A I G L L L L G L L L
631 L L L A P L L L L T C D C G A G S T G G V T G G F I P V P D
661 G S E G T I H Q W G I E G A H P E D K E I T N I C V P P V T
691 A N G A D F M E S S E V C T N T Y A R G T A V E G T S G M E
721 M T T K L G A A T E S G G A A G F A T G T V S G A A S G F G
751 A A T G V G I C S S G Q S G T M R T R H S T G G T N K D Y A
781 D G A I S M N F L D S Y F S Q K A F A C A E E D D G Q E A N
811 D C L L I Y D N E G A D A T G S P V G S V G C C S F I A D D
841 L D D S F L D S L G P K F K K L A E I S L G V D G E G K E V
871 Q P P S K D S G Y G I E S C G H P I E V Q Q T G F V K C Q T
901 L S G S Q G A S A L S A S G S V Q P A V S I P D P L Q H G N
931 Y L V T E T Y S A S G S L V Q P S T A G F D P L L T Q N V I
961 V T E R V I C P I S S V P G N L A G P T Q L R G S H T M L C
991 T E D P C S R L I
```

18. An antibody to the peptide having the amino acid sequence:

```
                5         10        15        20        25        30
                |         |         |         |         |         |
  1 M M G L F P R T T G A L A I F V V V I L V H G E L R I E T K
 31 G Q Y D E E E M T M Q Q A K R R Q K R E W V K F A K P C R E
 61 G E D N S K R N P I A K I T S D Y Q A T Q K I T Y R I S G V
 91 G I D Q P P F G I F V V D K N T G D I N I T A I V D R E E T
121 P S F L I T C R A L N A Q G L D V E K P L I L T V K I L D I
151 M D N P P V F S Q Q I F M G E I E E N S A S N S L V M I L N
181 A T D A D E P N H L N S K I A F K I V S Q E P A G T P M F L
211 L S R N T G E V R T L T N S L D R E Q A S S Y R L V V S G A
241 D K D G E G L S T Q C E C N I K V K D V N D N F P M F R D S
271 Q Y S A R I E E N I L S S E L L R F Q V T D L D E E Y T D N
301 W L A V Y F F T S G N E G N W F E I Q T D P R T N E G I L K
331 V V K A L D Y E Q L Q S V K L S I A V K N K A E F H Q S V I
361 S R Y R V Q S T P V T I Q V I N V R E G I A F R P A S K T F
391 T V Q K G I S S K K L V D Y I L G T Y Q A I D E D T N K A A
421 S N V K Y V M G R N D G G Y L M I D S K T A E I K F V K N M
451 N R D S T F I V N K T I T A E V L A I D E Y T G K T S T G T
481 V Y V R V P D F N D N C P T A V L E K D A V C S S S P S V V
511 V S A R T L N N R Y T G P Y T F A L E D Q P V K L P A V W S
541 I T T L N A T S A L L R A Q E Q I P P G V Y H I S L V L T D
571 S Q N N R C E M P R S L T L E V C Q C D N R G I C G T S Y P
601 T T S P G T R Y G R P H S G R L G P A A I G L L L L G L L L
631 L L L A P L L L L T C D C G A G S T G G V T G G F I P V P D
661 G S E G T I H Q W G I E G A H P E D K E I T N I C V P P V T
691 A N G A D F M E S S E V C T N T Y A R G T A V E G T S G M E
721 M T T K L G A A T E S G G A A G F A T G T V S G A A S G F G
751 A A T G V G I C S S G Q S G T M R T R H S T G G T N K D Y A
781 D G A I S M N F L D S Y F S Q K A F A C A E E D D G Q E A N
811 D C L L I Y D N E G A D A T G S P V G S V G C C S F I A D D
841 L D D S F L D S L G P K F K K L A E I S L G V D G E G K E V
871 Q P P S K D S G Y G I E S C G H P I E V Q Q T G F V K C Q T
901 L S G S Q G A S A L S A S G S V Q P A V S I P D P L Q H G N
931 Y L V T E T Y S A S G S L V Q P S T A G F D P L L T Q N V I
961 V T E R V I C P I S S V P G N L A G P T Q L R G S H T M L C
991 T E D P C S R L I
```

19. A method for the diagnosis of pemphigus vulgaris disease comprising the steps of:

(i) coating a surface with all, or a unique portion, of the pemphigus vulgaris antigen according to claim 17;

(ii) contacting said coated surface with serum from an individual suspected of having said disease; and

(iii) detecting the presence or absence of a complex formed between said pemphigus vulgaris antigen and antibodies specific therefor present in said serum.

20. A diagnostic kit comprising a recombinantly produced pemphigus vulgaris antigen and ancillary reagents suitable for use in detecting the presence of antibodies to said pemphigus vulgaris antigen in a mammalian serum or tissue sample.

21. A therapeutic method for the treatment of pemphigus vulgaris disease comprising performing plasmapheresis on an individual having pemphigus vulgaris disease, wherein the pemphigus vulgaris antigen according to claim 17 is contacted with the individual's blood prior to reinfusion of the blood into the individual.

FIG. 1

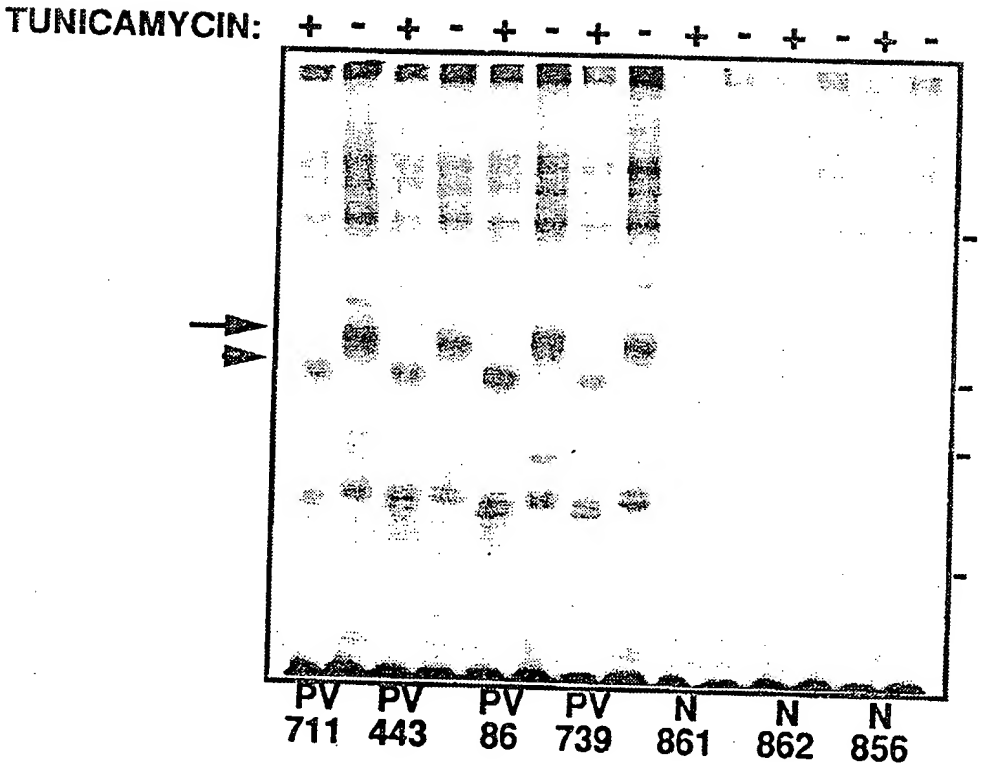


FIG. 2A FIG. 2B

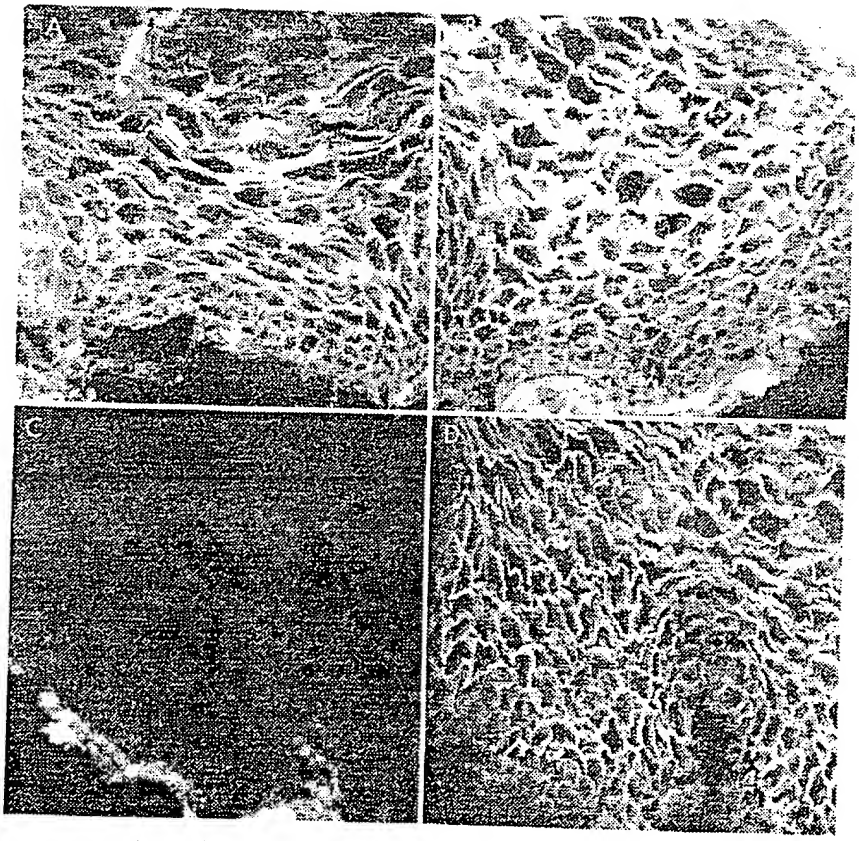


FIG. 2C FIG. 2D

FIG. 3

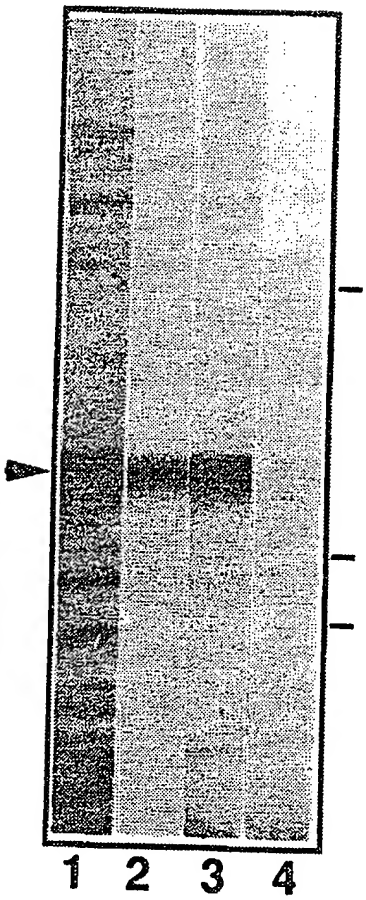


FIG. 4

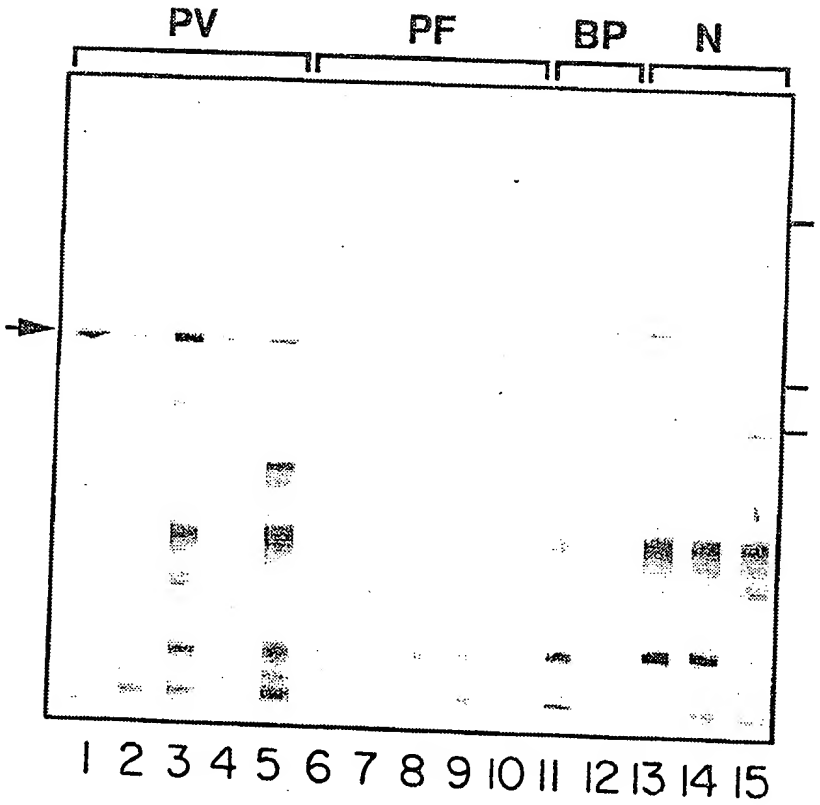


FIG. 5

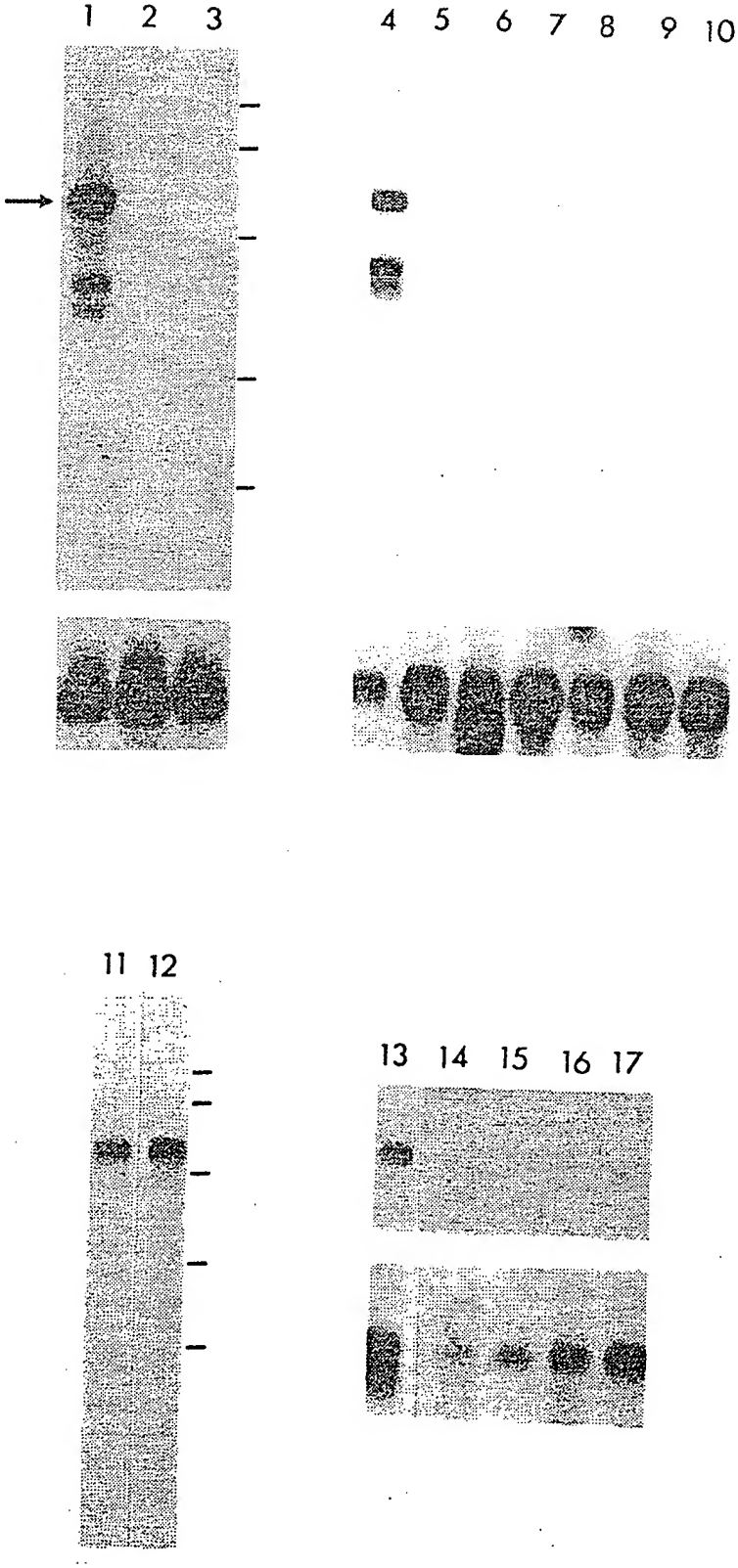


FIG. 6

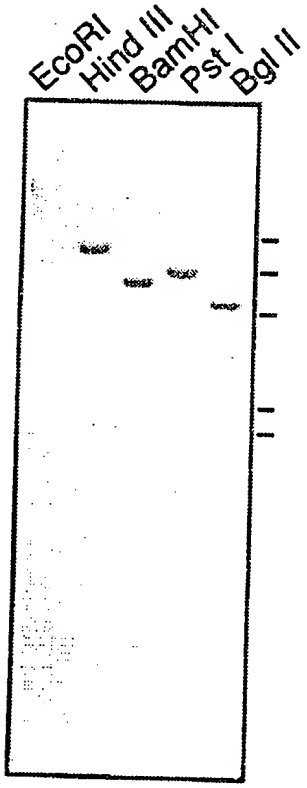


FIG. 7A


→E33
1 TTTTCTTAGACATTAACTGCAGACGGCTGGC
AGGATAGAAGCAGCGGCTCACTTGGACTTTTTCACCAGGGAAATCAGAGACA
83 ATGATGGGGCTCTTCCCCAGAACTACAGGGGCTCTGGCCATCTTCGTGGTGG
1 M M G L F P R T T G A L A I F V V
TCATATTGGTTCATGGAGAATTGCGAATAGAGACTAAAGGTCAATATGAT
V I L V H G E L R I E T K G Q Y D
185 GAAGAAGAGATGACTATGCAACAAGCTAAAAGAAGGCAAAAACGTGAATGGG
34 E E E M T M Q Q A K R R Q K R E W
→ EC1
TGAAATTTGCCAAACCCTGCAGAGAAGGAGAAGATAACTCAAAAAGAAAC
V K F A K P C R E G E D N S K R N
287 CCAATTGCCAAGATTACTTCAGATTACCAAGCAACCCAGAAAATCACCTACC
68 P I A K I T S D Y Q A T Q K I T Y
GAATCTCTGGAGTGGGAATCGATCAGCCGCCTTTTGGAATCTTTGTTGTT
R I S G V G I D Q P P F G I F V V
389 GACAAAAACACTGGAGATATTAACATAACAGCTATAGTCGACCGGGAGGAAA
102 D K N T G D I N I T A I V D R E E
↑
CTCCAAGCTTCCTGATCACATGTCGGGCTCTAAATGCCCAAGGACTAGAT
T P S F L I T C R A L N A Q G L D

FIG. 7B

```
491 GTAGAGAAACCACTTATACTAACGGTTAAAATTTTGGATATTAATGATAATC
136  V  E  K  P  L  I  L  T  V  K  I  L  D  I  N  D  N
     CTCCAGTATTTTCACAACAAATTTTCATGGGTGAAATTGAAGAAAATAGT
     P  P  V  F  S  Q  Q  I  F  M  G  E  I  E  E  N  S
                → EC2

593 GCCTCAAACTCACTGGTGATGATACTAAATGCCACAGATGCAGATGAACCAA
170  A  S  N  S  L  V  M  I  L  N  A  T  D  A  D  E  P
                                  ↑
     ACCACTTGAATTCTAAAATTGCCTTCAAAATTGTCTCTCAGGAACCAGCA
     N  H  L  N  S  K  I  A  F  K  I  V  S  Q  E  P  A

695 GGCACACCCATGTTCCTCCTAAGCAGAAACACTGGGGAAGTCCGTACTTTGA
204  G  T  P  M  F  L  L  S  R  N  T  G  E  V  R  T  L
     CCAATTCTCTTGACCGAGAGCAAGCTAGCAGCTATCGTCTGGTTGTGAGT
     T  N  S  L  D  R  E  Q  A  S  S  Y  R  L  V  V  S

797 GGTGCAGACAAAGATGGAGAAGGACTATCAACTCAATGTGAATGTAATATTA
238  G  A  D  K  D  G  E  G  L  S  T  Q  C  E  C  N  I
     AAGTGAAAGATGTCAACGATAACTTCCCAATGTTTAGAGACTCTCAGTAT
     K  V  K  M  V  N  D  N  F  P  M  F  R  D  S  Q  Y
                                        → EC3
                              →E12
899 TCAGCACGTATTGAAGAAAATATTTTAAGTTCTGAATTACTTCGATTTCAAG
272  S  A  R  I  E  E  N  I  L  S  S  E  L  L  R  F  Q
     TAACAGATTTGGATGAAGAGTACACAGATAATTGGCTTGCAGTATATTTC
     V  T  D  L  D  E  E  Y  T  D  N  W  L  A  V  Y  F
```

FIG. 7C

FIG. 7D

```
1511 TCTACAGGCACGGTATATGTTAGAGTACCCGATTTCAATGACAATTGTCCAA
 476  S  T  G  T  V  Y  V  R  V  P  D  F  N  D  N  C  P
     CAGCTGTCCTCGAAAAAGATGCAGTTTGCAGTTCTTCACCTTCCGTGGTT
     T  A  V  L  E  K  D  A  V  C  S  S  S  P  S  V  V
     → EC5

                                                MJ315 ←
1613 GTCTCCGCTAGAACACTGAATAATAGATACACTGGCCCCTATACATTTGCAC
 510  V  S  A  R  T  L  N  N  R  Y  T  G  P  Y  T  F  A
     TGGAAGATCAACCTGTAAAGTTGCCTGCCGTATGGAGTATCACAACCCTC
     L  E  D  Q  P  V  K  L  P  A  V  W  S  I  T  T  L

1714 AATGCTACCTCGGCCCTCCTCAGAGCCCAGGAACAGATACCTCCTGGAGTAT
 544  N  A  T  S  A  L  L  R  A  Q  E  Q  I  P  P  G  V
      ↑
     ACCACATCTCCCTGGTACTTACAGACAGTCAGAACAATCGGTGTGAGATG
     Y  H  I  S  L  V  L  T  D  S  Q  N  N  R  C  E  M

1817 CCACGCAGCTTGACACTGGAAGTCTGTCAGTGTGACAACAGGGGCATCTGTG
 578  P  R  S  L  T  L  E  V  C  Q  C  D  N  R  G  I  C
     GAACTTCTTACCCAACCACAAGCCCTGGGACCAGGTATGGCAGGCCGCAC
     G  T  S  Y  P  T  T  S  P  G  T  R  Y  G  R  P  H
```

FIG. 7E


1919 TCAGGGAGGCTGGGGCCTGCCGCCATCGGCCTGCTGCTCCTTGGTCTCCTGC
612 S G R L G P A A I G L L L L G L L
TGCTGCTGTTGGCCCCCCTTCTGCTGTTGACCTGTGACTGTGGGGCAGGT
L L L L A P L L L L T C D C G A G
→ IA
2021 TCTACTGGGGGAGTGACAGGTGGTTTTATCCCAGTTCCTGATGGCTCAGAAG
646 S T G G V T G G F I P V P D G S E
GAACAATTCATCAGTGGGGAATTGAAGGAGCCCATCCTGAAGACAAGGAA
G T I H Q W G I E G A H P E D K E
2123 ATCACAAATATTTGTGTGCCTCCTGTAACAGCCAATGGAGCCGATTTCATGG
680 I T N I C V P P V T A N G A D F M
AAAGTTCTGAAGTTTGTACAAATACGTATGCCAGAGGCACAGCGGTGGAA
E S S E V C T N T Y A R G T A V E
→ C1
2235 GGCACTTCAGGAATGGAAATGACCACTAAGCTTGGAGCAGCCACTGAATCTG
714 G T S G M E M T T K L G A A T E S
GAGGTGCTGCAGGCTTTGCAACAGGGACAGTGTCAGGAGCTGCTTCAGGA
G G A A G F A T G T V S G A A S G
2327 TTCGGAGCAGCCACTGGAGTTGGCATCTGTTCCTCAGGGCAGTCTGGAACCA
748 F G A A T G V G I C S S G Q S G T
TGAGAACAAGGCATTCCACTGGAGGAACCAATAAGGACTACGCTGATGGG
M R T R H S T G G T N K D Y A D G

FIG. 7F

```
2429 GCGATAAGCATGAATTTTCTGGACTCCTACTTTTCTCAGAAAGCATTTGCCT
 782  A  I  S  M  N  F  L  D  S  Y  F  S  Q  K  A  F  A
      GTGCGGAGGAAGACGATGGCCAGGAAGCAAATGACTGCTTGTTGATCTAT
      C  A  E  E  D  D  G  Q  E  A  N  D  C  L  L  I  Y

2531 GATAATGAAGGCGCAGATGCCACTGGTTCTCCTGTGGGCTCCGTGGGTTGTT
 816  D  N  E  G  A  D  A  T  G  S  P  V  G  S  V  G  C
      GCAGTTTTATTGCTGATGACCTGGATGACAGCTTCTTGGACTCACTTGGA
      C  S  F  I  A  D  D  L  D  D  S  F  L  D  S  L  G

2633 CCCAAATTTAAAAAACTTGCAGAGATAAGCCTTGGTGTTGATGGTGAAGGCA
 850  P  K  F  K  K  L  A  E  I  S  L  G  V  D  G  E  G
                                                  → C2
      AAGAAGTTCAGCCACCCTCTAAAGACAGCGGTTATGGGATTGAATCCTGT
      K  E  V  Q  P  P  S  K  D  S  G  Y  G  I  E  S  C

2735 GGCCATCCCATAGAAGTCCAGCAGACAGGATTTGTTAAGTGCCAGACTTTGT
 884  G  H  P  I  E  V  Q  Q  T  G  F  V  K  C  Q  T  L
      CAGGAAGTCAAGGAGCTTCTGCTTTGTCCGCCTCTGGGTCTGTCCAGCCA
      S  G  S  Q  G  A  S  A  L  S  A  S  G  S  V  Q  P

2837 GCTGTTTCCATCCCTGACCCTCTGCAGCATGGTAACTATTTAGTAACGGAGA
 918  A  V  S  I  P  D  P  L  Q  H  G  N  Y  L  V  T  E
               → C3
      CTTACTCGGCTTCTGGTTCCCTCGTGCAACCTTCCACTGCAGGCTTTGAT
      T  Y  S  A  S  G  S  L  V  Q  P  S  T  A  G  F  D
```

FIG. 7G

```
2939 CCACTTCTCACACAAAATGTGATAGTGACAGAAAGGGTGATCTGTCCCATTT
 252  P  L  L  T  Q  N  V  I  V  T  E  R  V  I  C  P  I
      CCAGTGTTCCTGGCAACCTAGCTGGCCCAACGCAGCTACGAGGGTCACAT
      S  S  V  P  G  N  L  A  G  P  T  Q  L  R  G  S  H

3041 ACTATGCTCTGTACAGAGGATCCTTGCTCCCGTCTAATATGACCAGAATGAG
 986  T  M  L  C  T  E  D  P  C  S  R  L  I  *
      CTGGAATACCACACTGACCAAATCTGGATCTTTGGACTAAAGTATTCAAA

3143 ATAGCATAGCAAAGCTCACTGTATTGGGCTAATAATTTGGCACTTATTAGCT
      TCTCTCATAAACTGATCACGATTATAAATTAAATGTTTGGGTTCATACCC
                                          E12 ←

3245 CAAAAGCAATATGTTGTCACTCCTAATTCTCAAGTACTATTCAAATTGTAGT
      AAATCTTAAAGTTTTTCAAAACCCTAAAATCATATTCGC
```

FIG. 8A

```
pv    KRRQKR
dg    IRRQKR
pc    LRRHKR

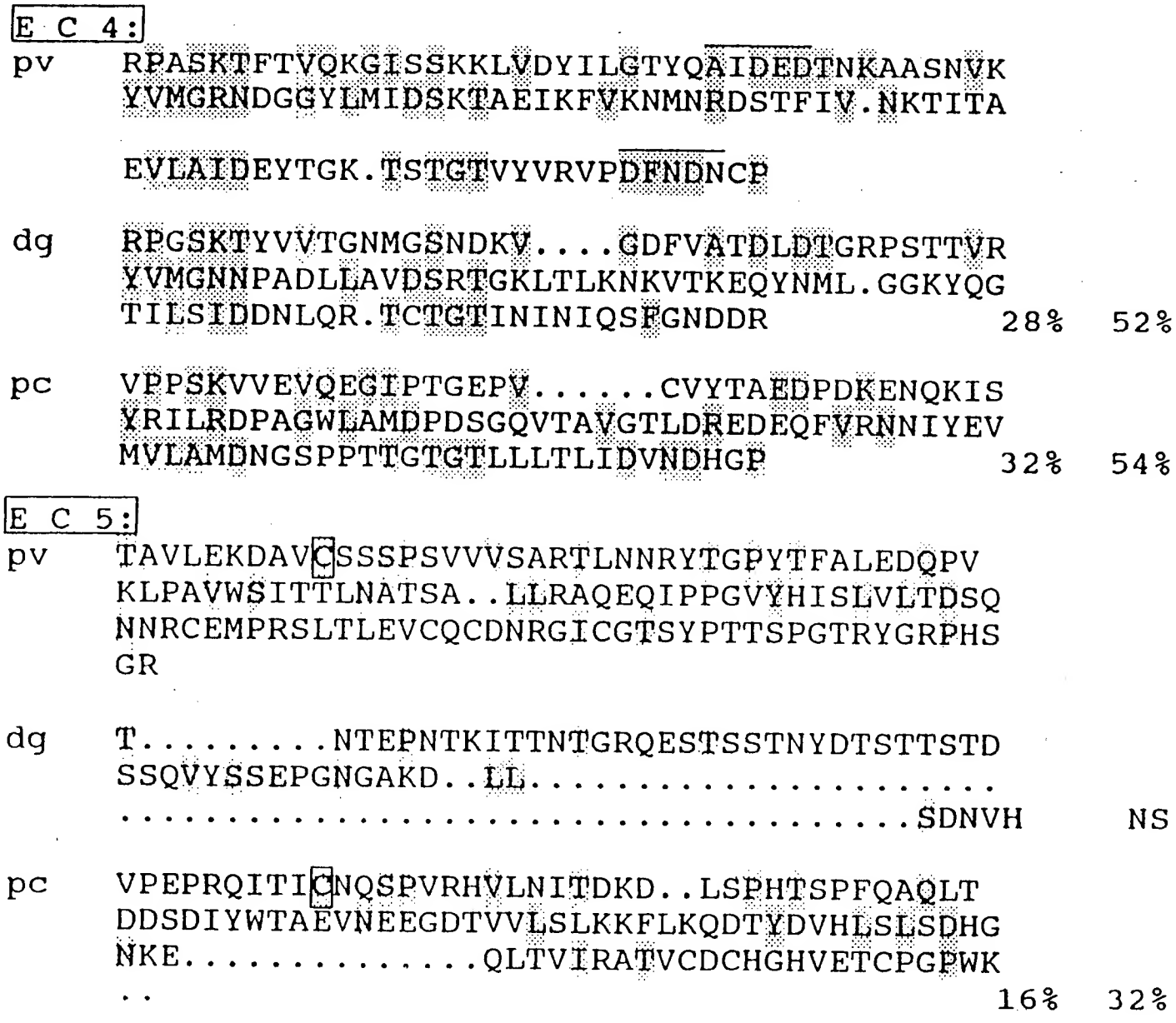
E C 1:
pv    EWVKFAKPCREGEDNSKRNPIAKITSDYQATQKITYRISG
                          ↓                  **
      VGIDQPPFGIFVVDKNTGDINITAIVDREETPSFLITCRA
      *
      LNAQGLDVEKPLILTVKILDINDNPPVF

dg    EWIKFAAACREGEDNSKRNPIAKIHSDCAANQQVTYRISG
      VGIDQPPYGIFVINQKTGEINITSIVDREVTPFFIIYCRA
      LNSMGQDLERPLELRVRVLDINDNPPVF          73%   82%

pc    DWVVAPISVPENGKGPFPQRLNQLKSNKDRDTKIFYSITGP
      GADSPPEGVFAVEKETGWLLLNKPLDREEIAKYELFGHAVS
      ENGASVEDPMNISIIVTDQNDHKPKF            31%   53%
```

FIG. 8B

FIG. 8C



SUBSTITUTE SHEET

FIG. 8D

T M:			
pv	..LGPAAIGLLLLGLLLLLLLAPLLLL		
dg	..FGPAGIGLLIMGFLVLGLVPFLMI	54%	92%
pc	GGFILPVLG.AVLALLFLLLVLLLLV	39%	78%
I A:			
pv	TCQQCGAGSTGGVTGGFIPVPDGSEGTIHQWGIEGAHPED		
	KEITNIC..VPPVTANGADFMESSEVCTNTYARGTAV		
dg	CCQQCGGAPRSA..AGFEPVPECSDGAIHSWAVEGPQPEP		
	RDITTVIPQIPPDNANIIECIDNSGVYTNEYG.GREM	37%	54%
pc	R.....KKRKIKEPLLLPEDDTRDNVFYYGEEGGGEED..		
	.....		NS

FIG. 8E

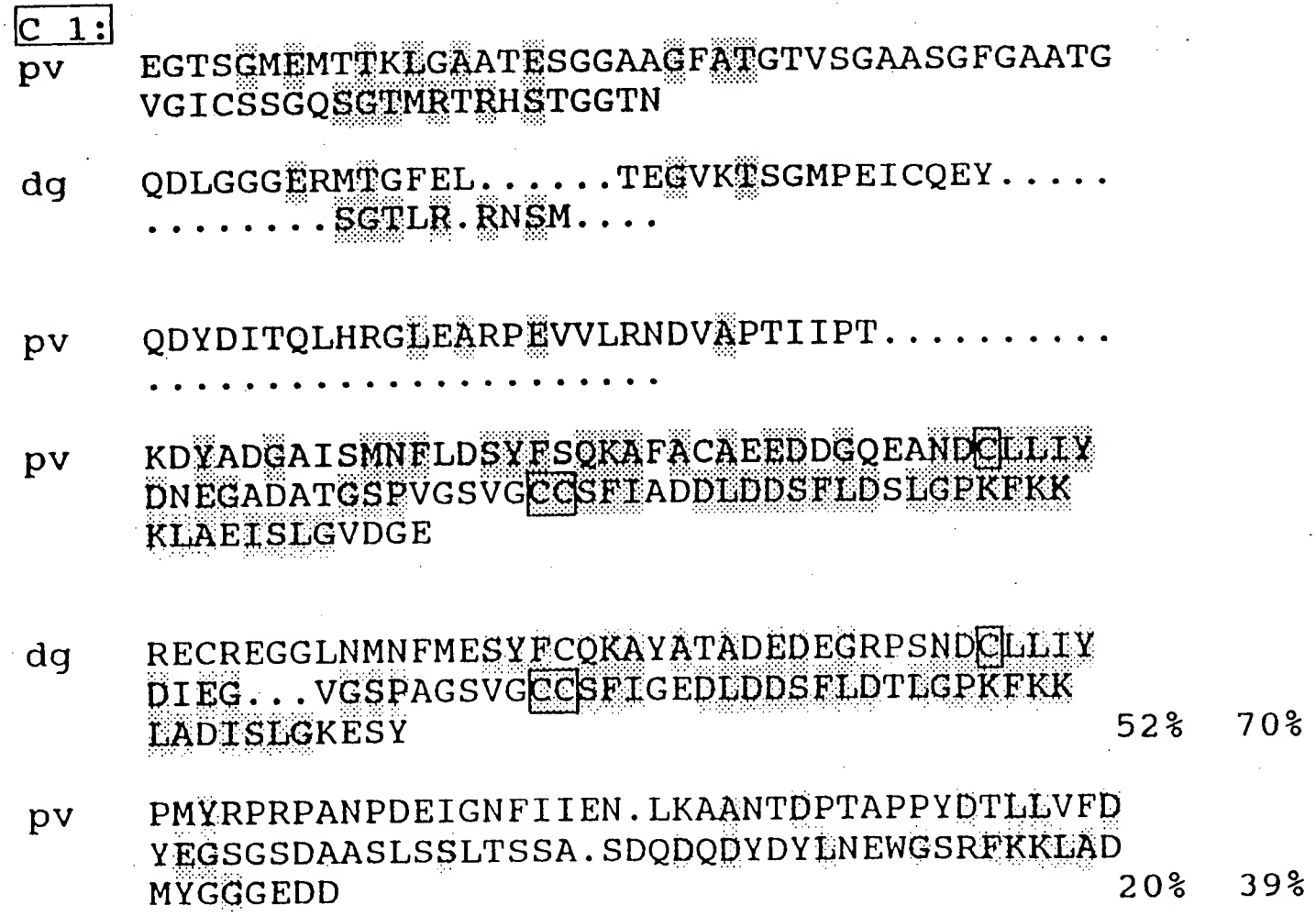
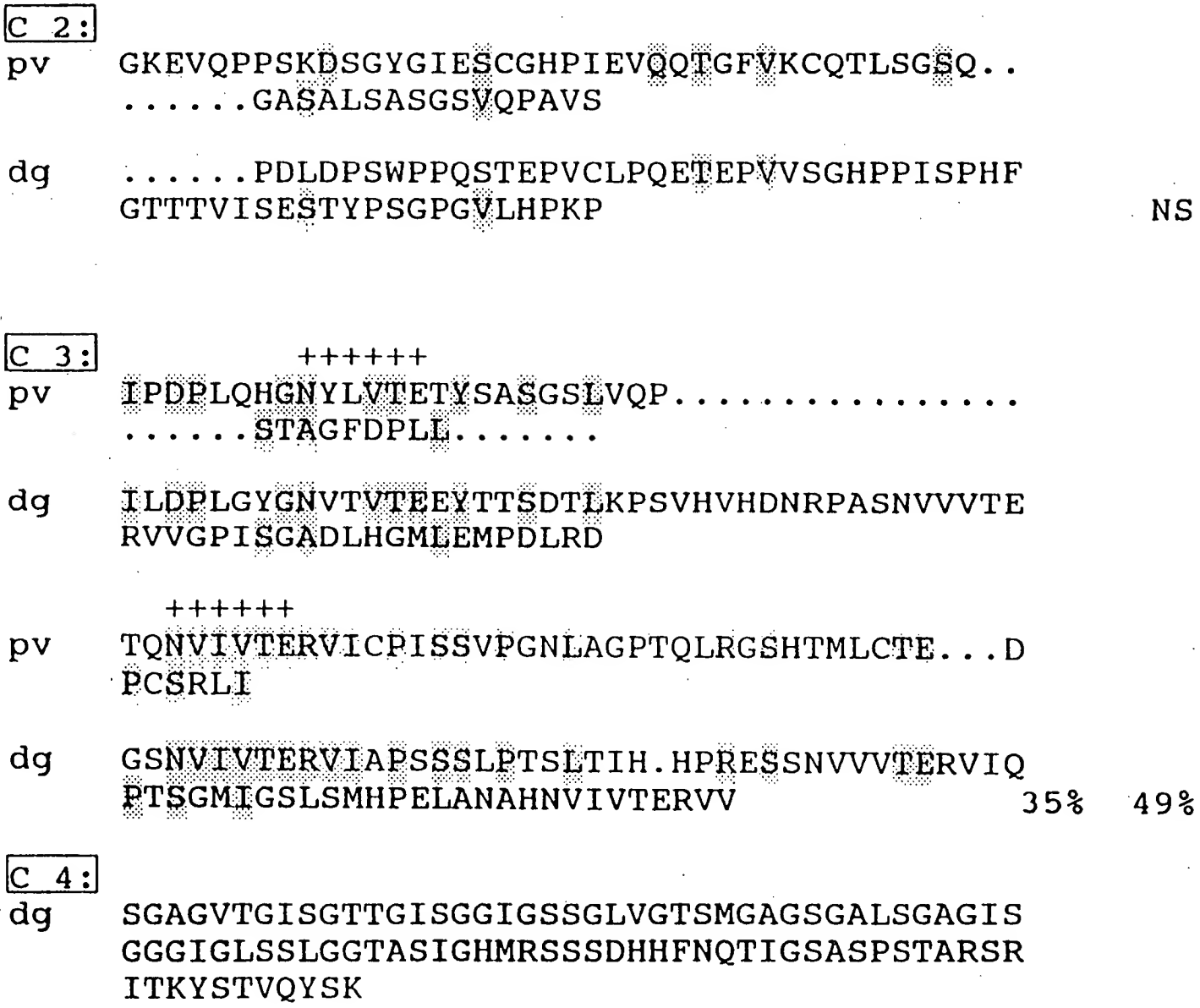

C 1:
pv EGTSGMEMTTKLGAATESGGAAGFATGTVSGAASGFGAATG
VGICSSGQSGTMRTRHSTGGTN
dg QDLGGGERMTGFEL.....TEGVKTSGMPEICQEY.....
.....SGTLR.RNSM....
pv QDYDITQLHRGLEARPEVVLRNDVAPTIIPT.....
.....
pv KDYADGAISMNFLDSYFSQKAFACAEEDDGQEANDCLLIY
DNEGADATGSPVGSVGCCSFIADDLDDSFLDSLGPKFKK
KLAEISLGVDGE
dg RECREGGLNMNFMESYFCQKAYATADEDEGRPSNDCLLIY
DIEG...VGSPAGSVGCCSFIGEDLDDSFLDTLGPKFKK
LADISLGKESY
52% 70%
pv PMYRPRPANPDEIGNFIIEN.LKAANTDPTAPPYDTLLVFD
YEGSGSDAASLSSLTSSA.SDQDQDYDYLNEWGSRFKKLAD
MYGGGEDD
20% 39%

FIG. 8F



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09933

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :424/88; 436/506; 435/69.3, 70.1, 7.21; 935/34
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88; 436/506; 435/69.3, 70.1, 7.21; 935/34

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, DERWENT, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF CLINICAL INVESTIGATIONS, Volume 74, issued August 1984, J.R. Stanley et al, "Distinction Between Epidermal Antigens Binding Pemphigus Vulgaris and Pemphigus Foliaceus Autoantibodies", pages 313-320, entire document.	1-21
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 83, issued October 1986, J.C.R. Jones et al, "A Cell Surface Desmosome-Associated Component: Identification of a Tissue-Specific Cell Adhesion Molecule", pages 7282-7286, entire document.	1-21
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 80, issued March 1983, R.A. Young et al, "Efficient Isolation of Genes by Using Antibody Probes", pages 1194-1198, see entire document	1-21
Y	D.M. Glover, "DNA CLONING VOLUME II A PRACTICAL APPROACH", published February 1986 by IRL Press (OXFORD, ENGLAND), pages 191-211, and 213-239, see entire document.	7,8,11,16-21
Y	G.J. TORTORA et al, "MICROBIOLOGY AN INTRODUCTION", published 1989 by Benjamin/Cummings (CA), pages 446-447, see entire document	18-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 JANUARY 1993	Date of mailing of the international search report 12 FEB 1993
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ANTHONY C. CAPUTA Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09933

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 39/00; G01N 33/564; C12P 21/06, 21/02; C12Q 1/00; G01N 33/53; C12N 15/00